"A STUDY ON THE RELATIONSHIP BETWEEN BLOOD LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND SEVERITY OF DIABETIC RETINOPATHY"

Dissertation submitted by

DR. JEBINTH BRAYAN

In partial fulfillment of the requirements for the degree of

MASTER OF SURGERY

IN

OPHTHALMOLOGY

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DEPARTMENT OF OPHTHALMOLOGY

PSG INSTITUTE OF MEDICAL SCIENCES & RESEARCH

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"Relationship between levels of vascular endothelial growth factor in blood and severity of diabetic retinopathy"

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2. Proposal
3. Informed Consent Forms
4. Proforma
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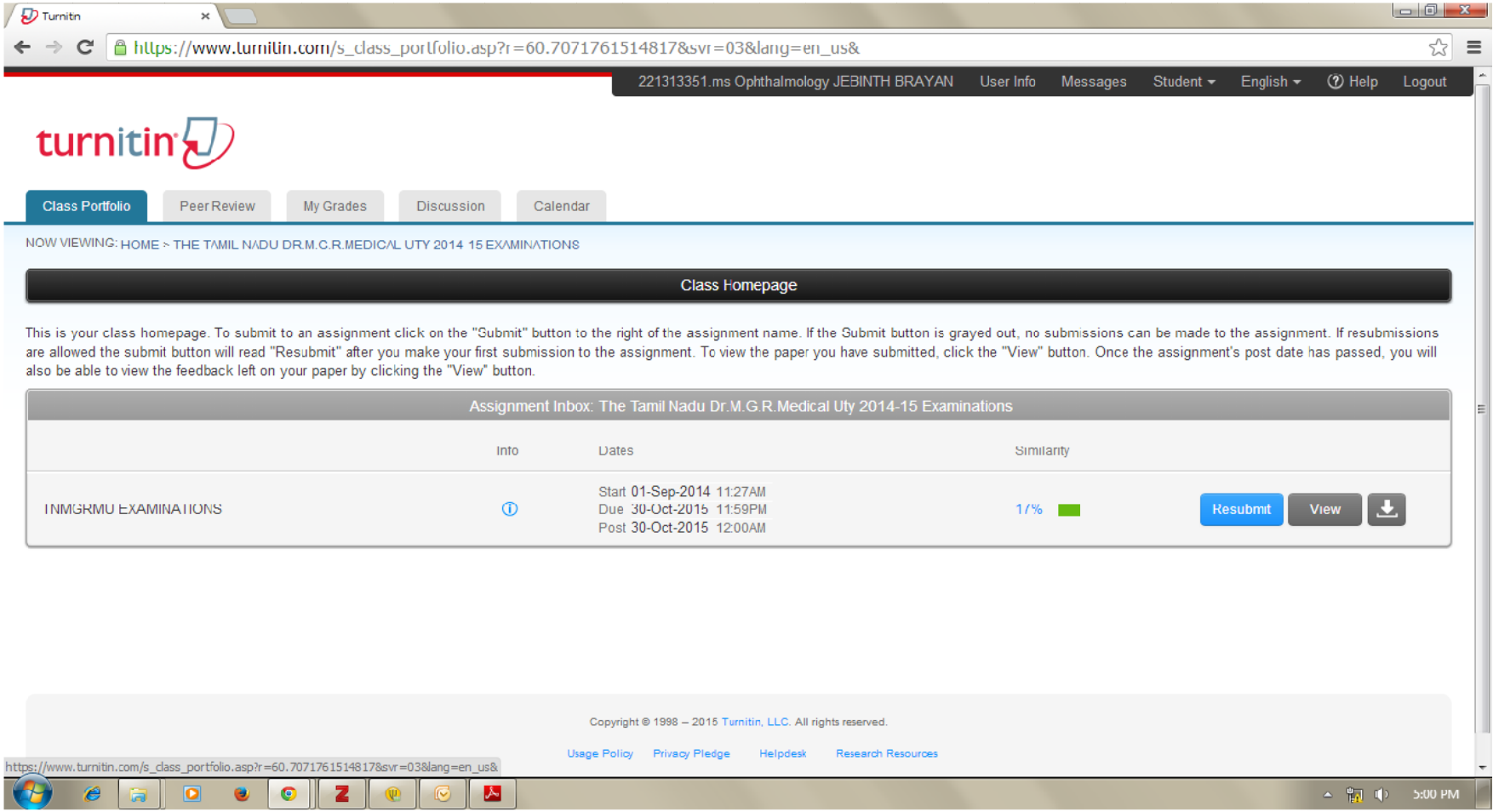
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“A STUDY ON THE RELATIONSHIP BETWEEN BLOOD LEVELS OF VASCULAR ENDOTHELIAL GROWTH FACTOR AND SEVERITY OF DIABETIC RETINOPATHY”

Dissertation by Dr. JEBINTH BRAYAN

ABSTRACT

BACKGROUND: The importance of vascular endothelial growth factor in (VEGF) the pathogenesis of diabetic retinopathy is evident from numerous studies demonstrating a significant increase in VEGF levels in ocular samples. However, a correlation between blood levels of VEGF and diabetic retinopathy has not been conclusively proven or disproven. We undertake this study to demonstrate the relationship between blood levels of VEGF and the severity of diabetic retinopathy.

METHODS: The study population consisted of 75 Type 2 diabetic patients attending our outpatient department for routine diabetic retinopathy screening. After obtaining informed consent, 5ml of blood was drawn from each patient and estimated for the levels of serum VEGF. The data thus obtained was correlated with the grade of retinopathy. Additional parameters studied were duration of diabetes, haemoglobin levels, blood urea, serum creatinine, fasting and random blood sugars and HBA1C levels.

RESULTS: There was a significant elevation of blood VEGF levels when compared to the normal population. But this elevation was seen in all the patients irrespective of whether they had retinopathy or not. There was no correlation detected between VEGF levels and the severity of diabetes. There was a positive association between anemia and the severity of retinopathy. The levels of urea and creatinine were elevated in the more severe grade of retinopathy. There was an overall poor control of sugars reflected both by the high levels of fasting and random sugars and the high HBA1C.

CONCLUSION: Levels of blood VEGF are elevated in diabetic patients regardless of whether they have diabetic retinopathy or not. There is no statistically significant relationship between blood levels of VEGF and the severity of retinopathy. VEGF, therefore could present a potential treatment and preventive strategy for not only diabetic retinopathy but diabetes and its complications in general.

Introduction

Diabetic retinopathy (DR) can be either a non-proliferative or a proliferative pathology of the retina. Either way, it is probably the most significant complication of a systemic disease in the eye. Over a period spanning about half a century, the world has witnessed remarkable progress in the field of diabetic retinopathy (DR) and its management. From the early days of the Arlie house classification for staging of diabetic retinopathy, we have now entered firmly into the realm of anti-vascular growth factor (anti-VEGF) therapy.

A review of the literature shows five major points of change in the field of DR. Firstly, diabetes and its complications have become a global problem; to the extent that DR has assumed the status of a global epidemic. Diabetic retinopathy has thus become the leading cause of blindness in the middle aged population. (1) Secondly, as proved by a number of randomized control trials, tight control of blood sugars and additional systemic co-morbidities play a major role in the disease progression. (2)(3). Thirdly, optical coherence tomography (OCT) has become a major component in DR staging and management. With the improved resolution of images, ophthalmologists can cut down on the use of fundus fluorescein angiography. Fourthly, timely retinal photocoagulation can protect the retina from developing end stage disease. And lastly, the role of vascular endothelial growth factor (VEGF) appears to be central to the pathogenesis of DR. Thus anti-VEGF therapy has become one of the first lines of treatment, especially of diabetic macular edema.

The importance of VEGF in the disease pathogenesis was realized by the observation that the vitreous cavity of patients with DR had higher levels of VEGF than those of controls. To the best of our knowledge, all studies thus far, are mainly based

on measurements of VEGF in the vitreous cavity. (4)(5)(6)(7)(8).We aim to study the serum levels of VEGF in diabetic patients with DR and also the correlation, if any, between serum VEGF and the grading of DR. If such a correlation is found to exist, we believe it would have undeniable implications in the prognostication, monitoring and treatment of a disease capable of causing significant damage to an individual, a family and a nation, not to mention the whole world.

Aim

To establish a relationship between the serum levels of vascular endothelial growth factor and the severity of Diabetic Retinopathy.

Objectives

Primary outcome

1. Determine the serum VEGF levels in the study population of 75 patients of type 2 diabetes mellitus.
2. Determine whether this level is significantly altered with respect to normal ranges
3. Determine a trend (if any) of the serum VEGF and severity of diabetic retinopathy.

Secondary outcome

1. Study the demographic characteristics of the study population.
2. Determine the relationship between the duration of diabetes and the severity of retinopathy.
3. Determine the relationship between urea, serum creatinine and the severity of retinopathy.
4. Determine the relationship between fasting and random blood glucose and the severity of retinopathy
5. Determine any association between HBA1C and the severity of diabetic retinopathy.

Review of literature

Diabetes Mellitus- a global epidemic

Every seven seconds a person dies of a diabetes related complication(9). In 1995, the prevalence of diabetes in the adult population was estimated to be 4%. It is estimated to become 5.4% by 2025. In absolute figures, the number of adult diabetics will rise from 135 million in 1995 to 300 million in 2025. (1) The bulk of this increase will be in the developing countries, where there is predicted to be a 170% increase in the number of diabetic patients. By 2025, more than 75% of these diabetics will be in developing countries. This is in contrast to the 62% reported in 1995. Data from a number of studies show that the diabetes epidemic will affect the middle and low economic countries, with these countries contributing as much as 77% of the diabetic population.(10)(11)(12)

The Global burden due to diseases in general, is showing a shifting trend. Whereas, previously the major disease burden was due to communicable diseases, the trend nowadays seems to be shifting to noncommunicable disease.(13)(14)(15)(16)(17) In a study conducted by Lozano et al, there was a marked increase in the deaths due to diabetes. In their study conducted to estimate the Global Burden of diseases, injuries and risk factor, they reported 1.3 million diabetes related deaths- an almost doubling of the numbers seen in 1990. (15) The disability adjusted life years(DALY) due to diabetes- a measure of the mortality due to a disease- is also on the rise. In a study, published in The Lancet 2013, there was a shift of the DALYs from communicable disease to non-communicable disease with a decrease in the number of premature deaths and an increase in persons living with disability. There was an increase in the burden due to a variety of diseases, diabetes being a major one.

Diabetes in India

The prevalence of diabetes among Asians has been rapidly increasing. As of 2007, more than 110 million diabetics were from Asia.(18) Extrapolating from the current data available, the countries with the largest number of diabetic patients at present and in 2025 will be India, China and the United States. (1) As quoted in the study by Ramachandran et al, the national prevalence of diabetes has already doubled and more in a number of countries in this region. (11)

Table 1. ***A review of data from South India,(19)(11)(20)***

	Prevalence		Percentage increase	
	Urban	Rural	Urban	Rural
1989	8.2%	2.4%		
2006	18.6%	9.2%	2.3%	3.8%

In a study conducted on an urban slum in North India, the prevalence of diabetes was 10.3%, with males having a prevalence of 11.2% and females a prevalence of 9.9%. (21)

One of the postulated reasons for the diabetes boom in countries such as India and China is said to be the rapid urbanization and economic growth in the recent years. This has in turn led to changes in the nutritional habits and an increasingly sedentary lifestyle. (10) Urbanization leads to a significant decrease in physical activity with a corresponding increase in the body mass index. (22); all being risk factors for the development and progression of diabetes.

Apart from the rising epidemic in Asian countries, the Asian diabetic seems to acquire diabetes at an earlier age and with a lower body mass index. (23),(11)(18) This

type of “metabolically obese” phenotype- normal body weight with increased abdominal adiposity- seems to be typical of the Asian. This, along with an increased susceptibility to gestational diabetes, poor nutrition in utero and overnutrition in adult years seems to be contributing to the diabetes epidemic, constituting a vicious cycle where “diabetes begets diabetes”. (24)(18)(21)(11)(10) This younger age of onset and a longer duration of disease accordingly increases the morbidity and mortality associated with diabetes substantially.(24)(18)

Regardless, a worldwide surveillance of diabetes and its complications is necessary for its prevention and progression and should be given urgent priority in all parts of the world, regardless of whether they are developing or developed.(16)(14).

Definition of Diabetes-

Diabetes Mellitus is an all-encompassing term to describe conditions of metabolic disturbance wherein the main feature is a chronic elevation in blood sugars. The chief cause is either an impaired insulin action or an impaired insulin secretion or both. (25)(26)

The gold standard for diagnosis of diabetes is a measurement of the glucose levels in venous plasma. For accurate measurement glycolysis must be inhibited in the sample as soon as the blood is drawn. This can be achieved in by either storing the blood tube in ice and centrifuging in 30 minutes, or by adding inhibitors of glycolysis into the tube such as citrate with fluoride. In our institution, we are using the former technique

The guidelines for a diagnosis of DM are (25)(26)(27)

- Random plasma glucose >= 200 mg/dl (>=11.1 mmol/l)

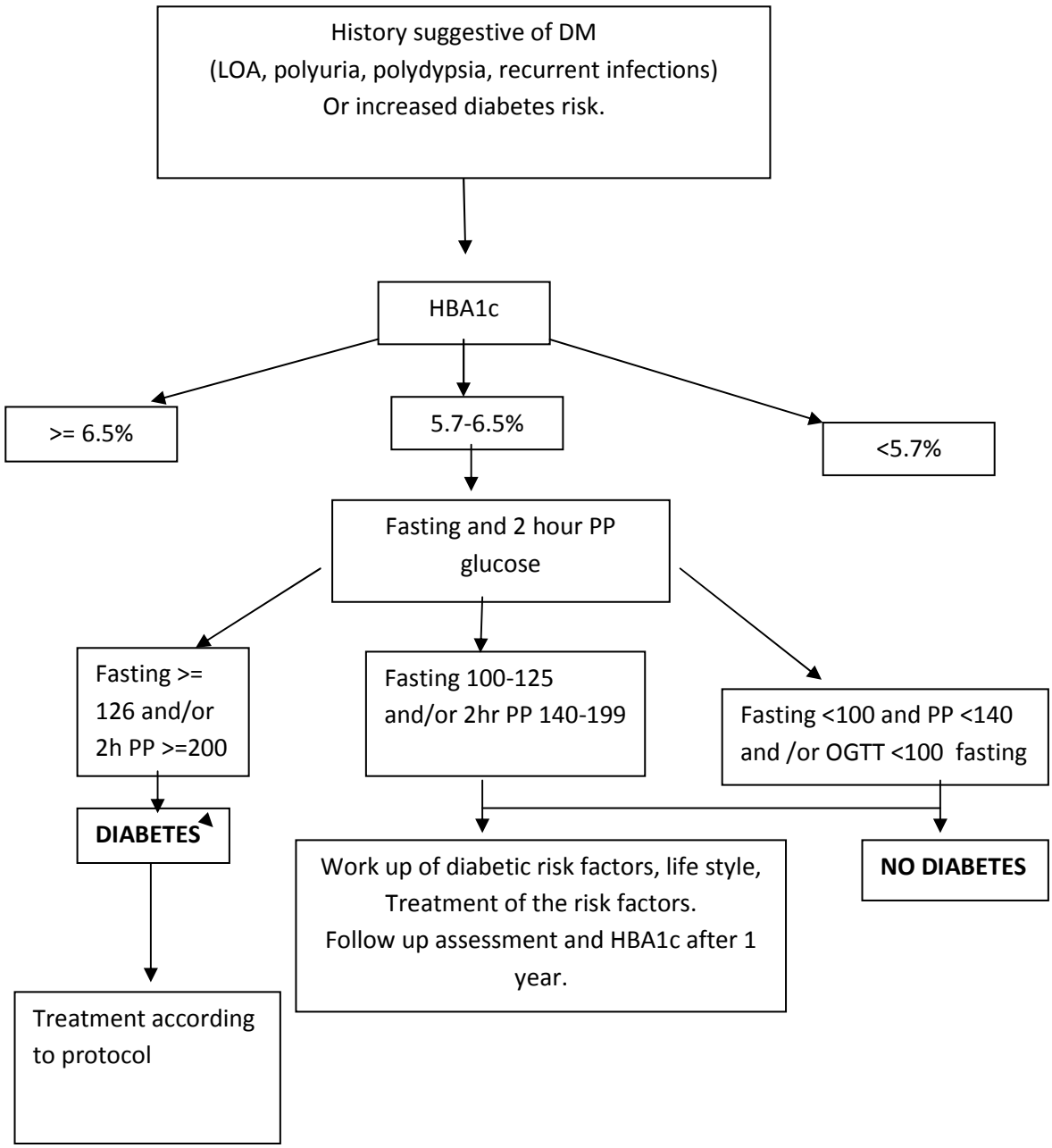
- Fasting >=126 mg/dl (>=7.0 mmol/dl)
- HbA1c >=6.5% (>= 48 mmol/mol)
- OGTT 2 hour glucose in venous plasma >=200 mg/dl (>= 11.1 mmol/l)

For a diagnosis to be made, persistant blood glucose levels meeting the diagnostic levels must be demonstrated on two or more occasions on two separate daysw.

A similar classification has also been proposed by the WHO. In addition, impaired glucose tolerance (IGT) have been assigned values that are above normal but below the diagnostic cutoffs for DM (plasma >= 6.1 to 7mmol/l)

The inclusion of HbA1c in the diagnostic process has been there since 2010. Recent studies have shown that the specificity of Hba1c levels >=6.5% is high enough for a diagnosis of diabetes and the sensitivity of HbA1c <5.7% is enough to exclude a diagnosis of diabetes; making HbA1c a useful primary screening tool in the diagnosis of diabetes.

Table 2. ***Flowchart for diagnosis of diabetes using HBA1c as the primary screening tool (25)***



Classification of DM (25)(26)(27)

Depending on the basic etiology, clinical features, age of onset and other factors DM can be broadly divided into the following

- Type 1 Diabetes

- B-cell destruction leading to an absolute deficiency in insulin
- Predominantly an immune mediated disease.
- Latent autoimmune diabetes in adults (LADA) is included in this category.

- Type 2 Diabetes

- Ranges from mainly an insulin resistance with a relative insulin deficiency to a defective insulin secretion along with insulin resistance.
- This type is very commonly associated with other problems constituting the metabolic syndrome.

- Other specific types of Diabetes

- Diabetes associated with disease of the pancreas (Pancreatitis, CF)
- Endocrine disorders (acromegaly, phaechromocytoma, cushings syndrome)
- Drug induced
- Genetic defects of the β-cells (MODY forms)
- Genetic defects of insulin activity
- Miscellaneous genetic syndromes which maybe associated with diabetes
- Infections
- Rare forms of auto-immune mediated diabetes.

- Gestational diabetes

Table 3. ***Differentiating features between Type 1 and Type 2 diabetes (25)***

	Type 1 Diabetes	Type 2 Diabetes
Age of onset	Mainly childhood, adolescents and young adults	Mainly middle and old age
Presentation	Usually acute to subacute onset	Usually gradual
Symptoms	Usually polyuria, polydipsia, weight loss and malaise	Often no complaints
Body weight	Often normal or thinly built	Frequently overweight
Progression to ketoacidosis	Marked	None or slight
Insulin secretion	Reduced or no secretion	Below normal to high, qualitatively always impaired
Insulin resistance	None (or only low)	Pronounced
Positive family history	Usually none	Typically positive
Concordance with identical twins	30-50%	Over 50%
Hereditary	Multifactorial	Multifactorial (likely to be polygenetic, but possible role for genetic heterogenicity)
Association with HLA system	Present	Not present
Antibodies associated with diabetic metabolism	Present in 90-95% at onset	None
Metabolism	Unstable	Stable
Response to insulin secretion stimulating antidiabetics	Usually none	Usually good at first
Insulin therapy	Needed	Usually not needed until insulin secretion has decreased after many years of the disease.

In addition to this classification, the report by the Japan Diabetes Society for the Classification and Diagnosis of Diabetes Mellitus advocate the importance of classifying the state of glycemia also, into normal, borderline and diabetic types.

- Diabetic type- fasting plasma glucose >=126 mg/dl and or 2 hour PP after a 75g glucose load >= 200mg/dl. A random glucose >=200 mg/dl is also suggestive of diabetic type.
- Normal type- fasting plasma glucose <110mg/dl and 2 hour PP <140 mg/dl
- Borderline type are patients who don't fall into either category. When OGTT is done, this type constitutes the sum of those with impaired fasting glycemia and impaired glucose tolerance. Patients with this type are more at risk for developing diabetes than the normal type. (27)(28)

<u>The complications of DM-</u>

The complications of DM are potentially disastrous and the importance of protecting the body from hyperglycemia cannot be overemphasized. These complications can be broadly divided into acute and chronic. The acute complications are chiefly due to severe hyperglycemia. This includes diabetic ketoacidosis and hyperosmolar nonketotic state. (29)

The majority of the chronic complications of diabetes involve the vascular tree and this forms the bulk of the morbidity and mortality associated with both type 1 and type 2 diabetes.(30)

Traditionally the chronic complications of diabetes have been divided into macrovascular and microvascular complications(29)(30)(31).

Table 4. ***Macro and microvascular complications of diabetes***

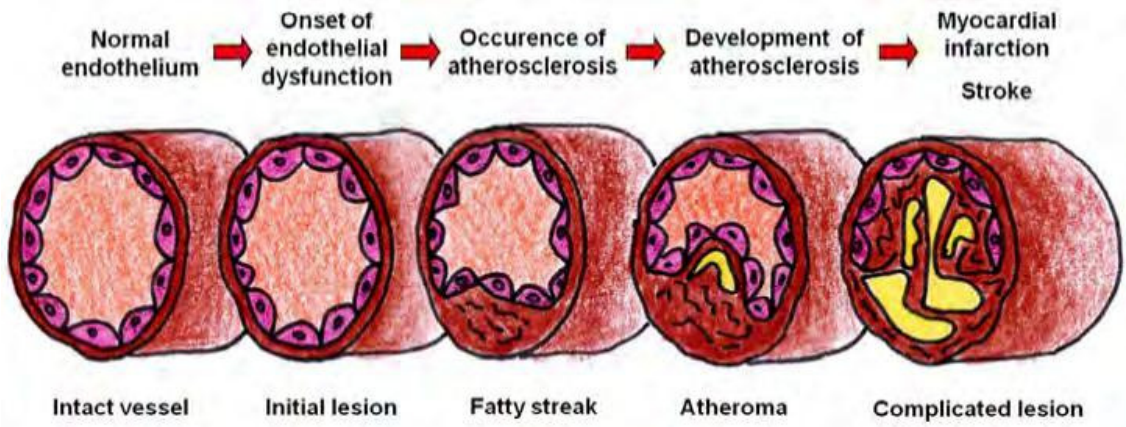
Macrovascular complications	
Coronary circulation	Myocardial ischeamia/infarction
Cerebral circulation	Transient ischeamic attack, stroke
Peripheral circulation	Claudication, ischemia
Microvascular complications/neuropathic	
Retinopathy, cataract	Impaired vision
Nephropathy	Renal failure
Peripheral neuropathy	Sensory loss Motor weakness
Autonomic neuropathy	Postural hypotension GI problem
Foot disease	Ulceration Arthropathy

Macrovascular complications of Diabetes-

The central pathology of the macrovascular disease is atherosclerosis. This effectively results in a narrowing of the arterial vasculature all over the body. (32) Chronic inflammation and intravascular injury to the arterial vasculature in the peripheral and cardiac circulations leads to atherosclerosis. As an effect of this endothelial damage, oxidized lipids from LDL particles get deposited in the endothelial layer of the arterial wall. Monocytes then migrate to the site and phagocytose the lipids to form foam cells. After foam cells are formed, they stimulate further macrophage migration and also attract T-lymphocytes. The T-lymphocytes, in turn, stimulate the formation and proliferation of smooth muscle cellsand collagen accumulatiom. Finally

all this leads to the formation of a lipid-rich atherosclerotic plaque in the arterial wall. Rupture of this leads to acute vascular infarction. (33)

Figure 1. ***Pathogenesis of arterial disease in DR***



The postulated link between Type 2 diabetes/insulin resistance and macrovascular disease includes a number of theories such as- reduced adiponectin concentration, increased formation of vascular cell adhesion molecule-1. These factors play a role in the T-lymphcyte adhesion to the endothelium. It also leads to a procoagulable state with increased expression of plasminogen activator inhibitor-1 (PAI-1) with additional atherosclerotic plaque instability. (33) The deadly combination of increased coagulability with impaired fibrinolysis increases the likelihood of vascular occlusion and vascular complications associated with DM type 2. (34)

The precise mechanism/s by which diabetes increases the risk of developing atheromatous plaque has not been clearly defined, the association between the two cannot be disputed.(30). There are a number of theories including the activation of the aldose reductase pathway, formation of an environment of elevated oxidative stress, advanced glycation end products, and the protein kinase c theory.

Of all the macrovascular complications, coronary heart disease is probably the most common cause of death and morbidity. This association between CAD and diabetes has been highlighted in a number of studies beginning with the Framingham study. (35) In fact, it has been shown in more recent studies that the risk of myocardial infarction in diabetic patients is equivalent to the risk in nondiabetics with one prior attack, promoting DM to a status of risk equivalent for CAD rather than just a risk factor. (36)(37).

DM is also a strong predictive risk factor for stroke and peripheral vascular disease. The risk of stroke in a diabetic is increased by 150-400%. (38). The frequency of stroke related complications such as dementia are likewise elevated in diabetics. (34).

As mentioned above, DM often is present in a setting of metabolic disturbance. This metabolic syndrome includes obesity mainly of the abdominal variety, hypertension, hyperlipidemia, and hypercoagulabilty- all factors which can aggravate and promote vascular disease. Even so, diabetes can be considered as an independent risk factor for the development of ischeamic heart disease, CVD and even death. (39)

Microvascular complications of diabetes mellitus

Diabetic nephropathy-

Diabetic nephropathy by definition is proteinuria >500mg in 24 hours. However it is often preceded by degrees of proteinuria. This microalbuminuria is defined as an albumin excretion of 30-299mg in 24 hours. If left untreated, this microalbuminuria progresses to proteinuria and then frank diabetic renal failure.

In a study done by Gross et al, about 7% of diabetics have microalbuminuria at presentation. (40). According to the results published by the UKPDS, microalbuminuria had an annual incidence of 2% in type 2 diabetics and the prevalence after 10 years was 25%. (40)(41)

The pathological changes induced by diabetes in the kidney are increase in glomerular basement membrane thickness, microaneurysm formation, formation of Kimmelsteil-Wilson bodies (mesangial nodules) the underlying mechanism by which diabetes causes this is probably similar to the mechanism of diabetic retinopathy.

Diabetic neuropathy

"The symptoms and/or signs of peripheral nerve dysfunction in diabetics after the exclusion of other causes" constitutes the definition of diabetic neuropathy by the American Diabetes Association. (42). The likelihood of developing neuropathy depends on both the degree and duration of elevated sugars. In addition, some individuals possess some genetic characteristics making them more susceptible.

The eitiopathogenesis of peripheral neuropathy has not been elucidated, but several theories exist such as the role of polyol accumulation, oxidative stress injury and the role of AGE's.

The peripheral neuropathy can take several forms- sensory, focal/multifocal and autonomic neuropathies.

Chronic sensorimotor, symmetric, polyneuropathy is the most commonly encountered type in diabetics. The patient can present with a variety of symptoms such as burning, "shock-like" sensations, tingling or just a numb feeling. But the type with just numbness can present with a non-healing ulcer. On examination there is a loss to light touch, vibration and temperature sensation. There is also a loss of the ankle

reflex.(43). Studies have shown that especially individuals with a loss of 10-g monofilament are at an increased risk of ulceration of the lower limb. (44).

Mononeuropathies have a typical sudden onset and can involve any nerve. The most common nerves affected are the median, ulnar and radial nerves. In addition cranial nerves can also be affected. In electrophysiological tests there is a decrease both in the amplitude and the conduction of a nerve impulse. Severe pain, muscle weakness and atrophy, usually in the thigh, is called diabetic amyotrophy, and maybe a manifestation of mononeuropathy.

Diabetic autonomic dysfunction can occur in almost any organ. It can manifest in a number of ways such as- gastroparesis, constipation, diarrhea, bladder and bowel problems, silent ischemia and even sudden death due to cardiac irregularities. Silent myocardial ischemia is a cause of significant mortality. (45).

Diabetic retinopathy

Global indices of diabetic retinopathy.

DR has now become a very real threat to the quality of life for millions a people worldwide.(46)(47) The increasing prevalence of DR globally, mirrors the increase in the prevalence of diabetes. The number of people above 40 years of age in America to be affected by DR by the year 2050, is estimated to be 3.4 million.(46) This translates to $492 million loss towards direct medical treatment. In addition there are added costs due to lost wages and time. (46)

Using pooled data from 35 studies on more than 20,000 people, Yau et al estimated that there are about 93 million people with DR, 17 million with proliferative diabetic retinopathy (PDR), 21 million with diabetic macular edema. (48). Among diabetics, the prevalence of any kind of DR was 34.6%, PDR was 7.0%, DME was

6.6% and VTDR 10.2%. In a study conducted in Caucasians aged more than 40 years with type 2 DM, showed an overall prevalence of 40% for any kind of DR and 8% for VTDR. (49).

Taking a closer look at the epidemiological studies, the susceptibility to DR seems to vary among different ethnic groups. Higher prevalence of DR has been reported among Mexican Americans than in non-hispanic white people. (50)(51)(52). However, other studies have shown a lower prevalence of DR among African Americans and Mexican Americans than in non-Hispanic whites. (53)(54). There are a number of possible factors which could explain this ethnic difference in the rate and susceptibility to DR. a possible genetic susceptibility, socio-economic differences and access to medical care. There has also been speculation about a role for racial differences in the effect of DR risk factors. (55)(56).

Interestingly, apart from the significant visual impairment caused by DR, evidence suggests that just the presence of DR increases the risk of systemic vascular complications such as CVA, CAD, heart failure and kidney disease. The converse also seems to be true. Diabetes duration, HbA1c levels and blood pressure all play an important part in DR, and this correlation applies all the way across mild to vision-threatening stages of DR. (57)(58)(59)(60)(61).

In the report by Yau et al, higher serum cholesterol levels were associated with elevated risk of diabetic macular edema. (48) This fact is further strengthened by another, that fenofibrate, a lipid-lowering agent, may retard the progression of DR.(62) The prevalence of DR is higher in Type 1 diabetics than in Type 2. This is independent of the duration of diabetes. (63)(64)

Figure 2. ***Epidemiological data from 35 studies on DR (48)***

Study	Country	Year of photo	T2DM (%)	Male (%)	Mean age (range)	Ethnicity (%)	Fundus photography						Grading method	
							Eyes/sub	Dilated	Mydriasis	Field	Deg	Stereo	DR	DME
T1DM only														
EDC	U.S.	1986–1988	0	50.6	27.6 (8–48)	98 EU, 2 AA	2	√	√	3	30	√	ETDRS	CSME
Fyn	Denmark	2007–2008	0	59.8	58.6 (37–88)	100 EU	2	√	√	9	45	X	ETDRS	CSME
New Jersey 725	U.S.	1993–1998	0	40.4	27.5 (3–60)	100 AA	2	√	√	7	30	√	EDTRS	Other[1]
Turin	Italy	2006–2008	0	53.0	29.5 (7–68)	100 EU	2	√	X	2	45	X	AAO	No data
T2DM only														
Aarhus	Denmark	2000	100	56.5	65.0 (32–90)	100 EU	2	√	√	2	60	X	EDTRS	CSME
ADDITION	Denmark	2003	100	56.5	63.8 (43–78)	100 EU	2	√	√	2	60	X	ETDRS†	CSME
CURES ES	India	2001–2002	100	44.8	50.8 (20–85)	100 AS	2	√	√	4	30	√	ETDRS	CSME
Funagata	Japan	2000–2002	100	57.3	67.1 (37–92)	100 AS	1	X	X	1	45	X	ETDRS	CSME
Hoorn	Netherlands	1989–1992	100	45.9	64.9 (50–76)	100 EU	2	√	√	2	45	X	Eurodiab	No data
Samutsakhon	Thailand	2007	100	28.3	59.2 (27–86)	100 AS	2	X	X	7	30	√	Other[2]	No data
San Luis Valley	U.S.	1984–1988	100	43.3	58.6 (22–75)	66 HI, 34 EU	2	√	√	3	30	√	ETDRS	CSME
UKADS	U.K.	2004–2007	100	53.1	64.3 (17–96)	59 EU, 41 AS	2	√	X	2	45	√	UK NSCG	UK NSCG
T1DM and T2DM														
AusDiab	Australia	1999–2000	96.5	51.4	63.0 (25–91)	92 EU, 5 AS	2	X	X	2	45	X	ETDRS	Other[3]
BDES	U.S.	1988–1990	88.3	44.4	65.8 (44–86)	99 EU	2	√	√	7	30	√	ETDRS	CSME
Handan	China	2006–2007	99.7	35.9	57.6 (30–83)	100 AS	2	√	√	2	45	X	ETDRS	CSME
LALES	U.S.	2000–2003	97.6	43.8	58.5 (40–90)	100 HI	2	√	√	7	30	√	ETDRS	CSME
San Antonio	U.S.	1985–1986	97.8	40.6	54.4 (31–70)	82 HI, 18 EU	2	√	√	7	30	√	ETDRS	No data
WESDR	U.S.	1980–1982	58.5	48.5	50.9 (3–97)	99 EU, 1 AA	2	√	√	7	30	√	EDTRS	CSME
DM type not reported but deduced from age at diagnosis*														
Andhra Pradesh	India	1996–2000	97.9*	52.4	55.0 (25–86)	100 AS	1	√	√	2	30	√	Other[4]	Other[4]
Beijing	China	2006	100*	41.6	64.9 (45–87)	100 AS	2	√	X	2	45	X	ETDRS	CSME
BES	U.S.	1985–1988	95.6*	37.4	62.7 (40–91)	57 AA, 43 EU	2	√	√	2	45	√	Other[2]	No data
CHS	U.S.	1997–1998	99.1*	46.5	78.0 (69–95)	75 EU, 25 AA	1	X	X	1	45	X	ETDRS	CSME
EUREYE	7 European‡	2000–2003	99.2*	51.0	72.9 (64–93)	100 EU	2	√	√	1	35	√	Other[5]	No data
Hisayama	Japan	1998	98.5*	56.9	65.8 (43–96)	100 AS	2	√	X	1	45	X	ETDRS	No data
MVIP	Australia	1992–1994	96.7*	55.8	65.6 (42–97)	100 EU	2	√	X	2	30	√	AAO	CSME
NHANES	U.S.	2005–2008	95.4*	50.1	62.4 (40–85)	39 EU, 30 AA, 20 HI	2	X	X	2	45	√	ETDRS	CSME
Proyecto VER	U.S.	1997–1999	96.5*	37.3	60.5 (40–88)	100 HI	2	√	√	4	30	√	EDTRS	CSME
SINDI	Singapore	2007–2010	97.6*	52.3	61.0 (43–84)	89 AS	2	√	X	2	45	X	ETDRS	Other[6]
SNDREAMS	India	2004–2006	99.2*	53.0	56.3 (40–85)	100 AS	2	√	√	7	30	√	AAO	CSME

Study	Country	Year of photo	T2DM (%)	Male (%)	Mean age (range)	Ethnicity (%)	Fundus photography						Grading method	
							Eyes/sub	Dilated	Mydriasis	Field	Deg	Stereo	DR	DME
DM type not reported and could not be deduced														
ARIC	U.S.	1993–1995	NR	47.5	60.8 (50–71)	64 EU, 36 AA	1	X	X	1	45	X	ETDRS	CSME
BMES	Australia	1992–1994	NR	53.0	67.9 (51–96)	97 EU, 2 AS	2	√	√	6	30	√	ETDRS	CSME
MESA	U.S.	2002–2004	NR	52.0	65.5 (46–86)	36 AA, 30 HI, 22 EU, 12 AS	2	X	X	2	45	X	EDTRS	Other[7]
Rotterdam	Netherlands	1990–1993	NR	39.4	72.9 (55–96)	96 EU, 4 O	2	√	√	1	35	√	Other[5]	No data
Shihpai	Taiwan	1999–2000	NR	61.1	71.7 (65–90)	100 AS	2	√	√	2	35	X	AAO	CSME
SiMES	Singapore	2004–2006	NR	43.3	62.6 (40–80)	100 AS	2	√	X	2	45	X	ETDRS	Other[6]

AA, African American; AAO, American Academy of Ophthalmology; AS, Asian; CSME, clinically significant macular edema; DM, diabetes mellitus; ETDRS, Early Treatment Diabetic Retinopathy Study; EU, Caucasian, European ancestry; Eyes/sub, eyes per subject; HI, Hispanic; NR, not reported and could not be deduced; O, others; UK NSCG, United Kingdom National Screening Committee guidelines. ADDITION, Anglo-Danish-Dutch study of Intensive Treatment in People with Screen-detected Diabetes in Primary Care; ARIC, Atherosclerosis Risk in Communities Study; Andhra Pradesh, Andhra Pradesh Eye Disease Study; AusDiab, Australian Diabetes, Obesity and Lifestyle Study; BDES, Beaver Dam Eye Study; BES, Baltimore Eye Survey; BMES, Blue Mountains Eye Study; Beijing, Beijing Eye Study; CHS, Cardiovascular Health Study; CURES ES, Chennai Urban Rural Epidemiology Study (Eye Study); EDC, Pittsburgh Epidemiology of Diabetes Complications Study; EUREYE; European Eye Study; Funagata, Funagata Study; Handan, Handan Eye Study; Hisayama, Hisayama Study; Hoorn, Hoorn Study; LALES, Los Angeles Latino Eye Study; MESA, Multiethnic Study of Atherosclerosis; MVIP, Melbourne Vision Impairment Project; NHANES, National Health and Nutrition Examination Survey; Proyecto VER, Proyecto Vision and Eye Research; Rotterdam, Rotterdam Study; SiMES, Singapore Malay Eye Study; SINDI, Singapore Indian Eye Study; SNDREAMS, Sankara Nethralaya Diabetic Retinopathy Epidemiology and Molecular Genetics Study; T1DM, type 1 diabetes; T2DM, type 2 diabetes; UKADS, UK Asian Diabetes Study; WESDR, Wisconsin Epidemiologic Study of Diabetic Retinopathy. *DM type not reported by study but could be deduced from provided information regarding subject's age and duration of diabetes: Type 1 diabetes was assumed if subject was aged less than 30 years at diagnosis; type 2 diabetes was assumed if subject was aged 30 years or older at diagnosis. †ETDRS includes modified WESDR, modified Airlie House, modified ETDRS. ‡7 European countries: Norway, Estonia, UK, France, Italy, Greece, Spain. [1]Other: Macular edema (ME) = retinal thickening within 1 disc diameter of center of macula or history of ME with history of photocoagulation confirmed by treating physician. [2]Other: Not reported. [3]Other: Hard exudates (HE) within 1 disc diameter of macula. [4]Other: Adapted from Olk RJ, Lee CM. *Diabetic Retinopathy: Practical Management*. Philadelphia: JB Lippincott, 1993:3–20. [5]Other: Graded for presence of microaneurysms (MA) and/or dot hemorrhages with ICD codes. [6]Other: ME = HE in the presence of MA and blot hemorrhage within 1 disc diameter from foveal center or presence of focal photocoagulation scars in the macular area. [7]Other: Clinically significant macular edema (CSME) = macular edema within 500 μm of foveal center, or if photocoagulation scars were present in the macular area.

Diabetic retinopathy in India-

The burden of visual impairment in India is large and increasing. It is estimated that 1-1.5% of the Indian population are blind.(65)(66)(67) With a population exceeding 1 billion, this actually translates into huge numbers. DR is fast becoming a significant cause of visual pathology in India. (68) In the study by Dandona et al, on an urban population, of the total population studied, 1.8% above 30 years of age, suffered from DR. in the same study, DR was present in 22.4% of diabetics, self- reporting. (69). In another report carried out in a diabetes centre in South India, 34.1% of patients were reported to have DR. (70)

Table 5*. Self reported diabetes and diabetic retinopathy- Dandona et al*

	Males	Females
Self-reported diabetes	9.44%	6.49%
Diabetic retinopathy	2.14%	1.49%

In the above quoted study, most of the patients who had DR had mild or moderate Non proliferative diabetic retinopathy (NPDR) (89.3%), severe NPDR and Proliferative diabetic retinopathy were relatively less common (10.7%). A decrease in vision in either eye due to DR was present in a tenth of those with DR. No eye was blind due to DR in the population sample studied. This is in contrast to studies from developed countries, where a higher incidence of blindness has been reported. (71). The authors theorize that this could be due to the fact that in India, the diabetics die faster due to lack of adequate medical services. Also, most of the patients in their study had DM diagnosed after 30 years of age. It is an established fact that chances of DR are more when the DM has been diagnosed less than 30 years of age.

A study conducted in Kerala via a questionnaire distributed to self-reporting diabetics reports a 26.8% prevalence. (72). In a report from Chennai, the prevalence was 20.8%. (73). Interestingly, the prevalence of DR among first time diagnosed diabetic patients was 5.1%- this is less than those reported by other Western studies where the prevalence of DR at the time of diagnosis was from 20-35%. (63)(74)(75). This seems to hold true even when the age factor has been removed, as has been shown in other studies, such as the Asian Young Diabetes Study, where they reported a lower prevalence of DR in Indians when compared to other Asian groups. (76)

Table 6. ***The difference in the prevalence of DR in different ethnic groups (77)***

Population	Place	Year	Total diabetic population	Prevalence of retinopathy in percentage
Chennai Urban Rural Epidemiology Study (CURES)-eye study 1	Chennai, India	2003	1715	17.6%
Los Angeles Latino Eye Study (LALES)	Los Angeles, USA	1999-2003	1217	46.9
The Liverpool Diabetic Study	Liverpool, UK	1998	395	33.6
Barbados eye study	Barbados, west Indies	1998	615	28.8
Blue Mountains Eye Study	Blue Mountain, Australia	1992-1994	252	29.0
Taiwan	Taiwan, china	1991	527	35.0
Beaver Dam Eye Study	Wisconsin, USA	1988-1990	410	35.1
Wisconsin Epidemiologic Study of Diabetic Retinopathy	Southern Wisconsin, USA	1980-82	1313	50.3%

The lower prevalence of DR in Indians, as seen in the above table, maybe due to an inherent ethnic difference. Another theory proposed is the Indian diet, which though high in carbohydrates, includes more vegetables, less fatty substances and more anti-oxidants and anti-inflammatory substances.

Risk factors for the development of diabetic retinopathy(77)

Systemic factors	Ocular factors
Gender	Posterior vitreous detachment
Duration of DM	Old chorioretinopathy
Level of glycaemic control	Cataract surgery
Hypertension	
Associated nephropathy	
Altered lipid profile	
Pregnancy	
Alcohol	
Aneamia	
Obesity	

Gender-

There are conflicting reports on the association between gender and DR. in the Chennai study, DR seems to be more common in males as compared to female with a ratio of 2:1. (70)

Duration of disease-

All studies highlight the fact that duration of disease is strongly associated with increased prevalence and severity of DR. This has been shown in a number of Indian studies as well. (73)(69). In the study by Dandona et al, they reported an 87.5% prevalence of DR with those having DM for more than 15 years versus 18.9% for those with DM for less than 15 years. Also, it has been reported that for every five year added years of diabetes, the risk of DR is increased by 1.89.

Glycaemic control-

The benefits of maintaining strict control over the levels of sugars on the development and progression of DR has been emphasized in numerous studies. (63)(2). In the WESDR study, there was a 12% prevalence of DR when HbA1c levels were <7% versus 40.7% when HbA1c >10, in addition the chances of developing PDR with more severe retinal changes at baseline increased with the HbA1c levels. (63). Intensive therapy of sugars reduced ocular pathology by 54%, decreased the progression of NPDR to PDR or severe PDR by 47% and the requirement for laser by 56%. In the study by Remal et al, the final visual prognosis after laser photocoagulation was also dependant on the sugar control.

Hypertension-

Hypertension can affect DR by impaired autoregulation and hyperperfusion (hemodynamic) and through vascular endothelial factor (VEGF). Ultimately it adds to the damage to the retinal capillary endothelial cells.(78) There is a worsening of DR by the added presence of hypertension. (79)(80). In the Indian scenario, hypertension has

not been conclusively shown to be a confounding factor for DR. But uncontrolled hypertension does effect DR. (73)

Renal disease-

A number of studies have demonstrated a relationship between microalbuminuria, proteinuria and DR. (81)(82). This synergy between retinopathy and renal angiopathy maybe due to hypertension, increase fibrinogen levels and increased lipids. (83). Data from South India show a proteinuria in 29.2% of patients with DR. Studies from North India show an association between microalbuminuria and DR. (84)(85)

Elevated serum lipids-

The risk of developing hard exudates is increased with elevated lipid levels. (86)(87)(3). Similar findings have been reported from studies done in India. Some have demonstrated a decrease in the size of perimacular hard exudates. This is possibly due to an increase in pipid peroxidation in plasma. Significantly, macular edema showed an association with high LDL levels. (88)(89).

Pregnancy-

In Western literature, pregnancy has been shown to cause rapid progression of DR. However, this effect seems to be transient and the overall risk of progression is not increased by pregnancy. (90). Risk factors for progression include duration of diabetes, glucose control, the degree of retinopathy and the presence of other premorbid conditions. (91)There is a lack of Indian data on the behavior of DR in pregnancy.

Alcohol-

Heavy and prolonged alcohol intake has been associated with DR progression. (92) (93). However, in another study by Moss et al showed no significant association. (94)

Anemia-

A report by Singh et al reported spontaneous regression by microaneurysms by correction of anemia.(95) It is possible that co-existing anemia worsens DR by delivering small amounts of oxygen to already ischemic retinal tissue. (96) As demonstrated by the ETDRS, anemia was a risk factor for developing DR.(97). Furthermore, patients with DR and anemia had a five fold risk or more of developing severe retinopathy than patients with higher hemoglobin.(96)

Obesity-

A number of western studies have shown a relationship with body mass index (BMI), sugar control and blood pressure control. (98)(99)(100). In contrast, in Indian studies, type 2 diabetics and DR seemed to have a lower BMI. Possibly the difference in ethnicity is involved in this difference(73).

Ocular factors-

There are a number of ocular pathologies which seem to have a protective effect on the development of DR.

- Posterior vitreous detachment may prevent PDR because an intact posterior vitreous is needed for retinal new vessels. (101) Therefore a good examination of the vitreous is invaluable for predicting the development of DR.

- High myopia with chorioretinal atrophy or extensive chorioretinopathy act like photocoagulation and reduce the metabolic needs of the retina, thereby protecting against development of DR. (102)
- Cataract surgery, on the other hand, increases the chances of the development and progression of DR. (103). As demonstrated by the Palakkad Eye Disease Survey, cataract surgery was one of the main causes for decreased vision in diabetic patients. (72).

Other factors

- Recent reports have demonstrated a link with DR and atherosclerosis, suggesting a common pathogenesis between the macro and microvascular complications of diabetes. (104). As reported by the CURES study, intima-media thickness and artery wall stiffening were significantly associated with DR. (105)
- There seems to be a role for oxidative stress. Hypoglutathionaemia, along with oxidative stress cause altered metabolism which has been postulated to be a mechanism for microangiopathy asswociated with DR. (106)(107).
- As mentioned earlier, there is a role for genetic susceptibility in the development of DR. That is most likely why some patients develop DR even after good control whereas other patients do not inspite of very poor control. (108)(109). A number of studies have shown a clustering of DR among siblings.

<u>Staging of DR-</u>

There are a number of systems of classification of the severity of DR. there is considerable overlap between the different systems. All the systems are based on the two basic pathologies causing vision loss in DR- retinopathy and maculopathy. The

main differences between the systems are related mainly to terminology assigned for various levels of severity.

Classification based on clinical findings alone-

Two of the classification systems will be discussed here.

The Airlie House system is the gold standard for staging of DR. However, it is a very rigorous system and is therefore probably best reserved for research studies. It is based on assessment of seven 30 degree stereoscopic photographs of the retina (the seven standard fields) and comparing each of the images with standard photographs.(110) A score is then given to each eye ranging from 10 (no retinopathy) to 85 (proliferative retinopathy). The grades for both eyes are then compared.

- Field 1- centered on the macula.
- Field 2- centered on the optic disc.
- Fields 3 to 8- two above, two below and one nasal to the disc surrounding the fields 1 and 2

The findings graded in fields 2 to 8 are haemorrages, microaneurysms, hard exudates, cotton wool spots, venous abnormalities (caliber abnormalities, sheathing, perivenous exudates), arteriolar abnormalities, intraretinal microvascular abnormalities (IRMA) and neovascularisation.

Table 7. ***Modified airlie house grading of diabetic retinopathy (111)*** **-**

Retinopathy level	Retinopathy severity	Retinopathy definition
10	No retinopathy	Diabetic retinopathy absent
20	Very mild NPDR	Microaneurysms only
35	Mild NPDR	Hard exudates, cotton-wool spots, and/or mild retinal hemorrhages
43	Moderate NPDR	43A:retinal hemorrhages moderate (>photograph 1A) in 4 quadrant or severe (≥ photograph 2A) in 1 quadrant 43B:mild IRMA (<photograph 8A) in 1 to 3 quadrants
47	Moderate NPDR	47A:both level 43 characteristics 47B:mild IRMA in 4 quadrants 47C:severe retinal hemorrhage in two to three quadrants 47D:venous beading in one quadrant"
53A-D	Severe NPDR	53A:≥2 level 47 characteristics 53B:severe retinal hemorrhages in 4 53C:moderate to severe IRMA (≥ photograph 8A) in at least 1 quadrant 53D:venous beading in at least 2 quadrants"
53E	Very severe NPDR	≥2 level 53A-D characteristics
61	Mild PDR	NVE <0.5 disk area in 1 or more quadrants
65	Moderate PDR	65A:NVE≥0.5 disk area in 1 or more quadrants 65B:NVD<photograph 10A (0.25-0.33 disk area)
71 and 75	High-risk PDR	NVD ≥ photograph 10A, or NVD < photograph 10A or NVE ≥ 0.5 disk area plus VH or PRH, or VH or PRH obscuring ≥ 1 disk area
81 and 85	Advanced PDR	Fundus partially obscured by VH and either new vessels ungradable or retina detached at the center of the macula

NPDR: Non proliferative diabetic retinopathy, PDR: Proliferative diabetic retinopathy, IRMA: Intraretinal microvascular abnormalities, NVE: New vessels elsewhere, NVD: New vessels on or within 1 DP of the optic disk, PRH: Pre-retinal hemorrhage, VH:Vitreous hemorrhage.

The ETDRS in addition, introduced the term clinically significant macular edema (CSME)(112). This was defined by three criteria(113)-

- Thickening of the retina at/or within 500 µm of the center of the macular
- Hard exudates within 500µm of the macular center along with adjacent retinal thickening.
- An area of retinal thickening 1 disc area, any part of which is within 1 disc diameter of the macular center.

In addition macular edema can be classified as focal and diffuse. (114). However, these terms do not have any strong treatment benefit.

In order to simplify the Airlie House classification, the International Clinical Disease Severity Scale for DR was introduced. This system is simple to use and easy to remember. There are five stages

- Stage 1- No apparent retinopathy
- Stage 2- mild non-proliferative diabetic retinopathy (mild NPDR): upto a few microaneurysms
- Stage 3- Moderate NPDR: presence of microaneurysms, intraretinal heamorrages or venous beading which do not reach the severity of sever NPDR (standard photographs 2A, 6A and 8A)
- Stage 4- Severe NPDR: 4:2:1 rule, any one of the following
  - heamorrages (of at least the magnitude of photograph 2A) in all 4 quadrants
  - venous beading (at least of magnitude photograph 6A) in 2 quadrants
  - IRMA (at least of magnitude photograph 8A) in even one quadrant
- Stage 5- Proliferative diabetic retinopathy (PDR): neovascularisation of the retina, disc, iris, angle, vitreous hemorrhage or tractional retinal detachment.

Some include a stage of very severe NPDR, this falls in between the stages of severe NPDR and PDR.

Macular edema is either present or absent. If it is present, it can be further graded as mild, moderate or severe as regards the distance between the thickening and the macular center. (115)

Classification based on fundus fluorescein angiography (FFA)-

The EDTRS proposed a system where there were stereoscopic FFA pictures of two 30 degree fields extending along the horizontal meridian from 25 degree nasal to the disc to 20 degree temporal to the macula. The pictures were assessed for early mid-phase capillary drop out, dilatation, arteriolar pathologies and also the size of the foveal avascular zone. (116). But this system is complicated and best suited for research.

Classification of DR based on Optical coherence tomography (OCT)-

This non-invasive, non-contact investigative modality is very useful for assessment of diabetic macular edema. With the rising importance of anti-VEGF therapy for diabetic macular edema, OCT is fast becoming an essential tool for the treatment of DR. (117)Several OCT morphological patterns are described for macular edema. These include diffuse retinal thickening, cystoids edema, exudative retinal detachment, tractional retinal detachment, posterior vitreous traction. (118). Unfortunately, there is no consensus till date on a grading system of macular edema based on OCT findings alone. (119)

Pathogenesis of DR-

It would not be an exaggeration to say that the pathogenesis of DR is extremely complex. There are a number of vascular, inflammatory and neuronal mechanisms involved. (120). Changes in retinal microvasculature are key to understanding the disease process. (121).

The disease can be simply understood as occurring in two phases. In the first phase, there is a compromise of retinal microvasculature which results in the retinal capillaries degenerating. This then leads on to an angiogenic over-compensation. The early alterations in the retinal microvasculature are a disruption of blood flow, a thickened basement membrane, loss of mural cells and formation of abnormal capillaries. Due to endothelial cell destruction and capillary loss there is a hypoxia of the inner retina. Simultaneously a number of growth factors and inflammatory substances-most of them being angiogenic- are secreted leading to the generation of abnormal preretinal vasculature. (122)

The important players of DR pathogenesis-

That inflammation and angiogenesis play a major role in DR is widely accepted. (123) Of course, the exact underlying mechanism and interactions have not yet been clearly elucidated. By studying aqueous and vitreous samples, fibrovascular tissue from retinae of eyes affected by DR and vitreous heamorrage, the following mediators appear to have key roles in the pathogenesis of DR.

Table 8. ***Inflammatory mediators of DR***

Vitreous mediators		Function
Cytokines	IL-6	Regulating immune reponses Increasing vascular permeability Angiogenesis Regulating expression of metalloproteinases
	IL-8	Chemoattractant Angiogenesis
	IL-1β	Angiogenesis Synthesizing collagen
	TNF-α	Antiangiogenic activity Leukocyte adhesion oxidation
	HMGB1	Stabilizing the formation of nucleosomes and gene transcription Attenuating retinal injury after ischemia-reperfusion Mediating the secretion of survival factors
Transcription factors	NF-κB	Regulating immune response, cell proliferation and apoptosis Synthesizing cytokines, chemokines and proinflammatory molecules
	HIF-1	Regulating cellular responses under acute and chronic hypoxia Regulating VEGF expression
Chemokines	MCP-1	Recruiting and activating macrophages Fibrosis and angiogenesis
	IP-10	Inhibiting angiogenesis
	MIG	Angiostatic activity
	SDF-1	Stimulating and mobilizing cells of tissue repair, promoting migration, proliferation and differentiation of endothelial cells Promoting repair after ischeamic injury Angiogenesis
	Fractalkine	Angiogenesis
	MIF	Recruiting and enhancing macrophages adherence, motility and phagocytosis

Growth factors	VEGF	Increasing vascular permeability Angiogenesis Endothelilal cell migration and survival Expression of ICAM and VCAM-1
	PGF	Potentiating the action of VEGF Stimulating endothelial cell proliferation, migration and angiogenesis
	Tenascin –C	Modulating cell growth and adhesion Sprouting of endothelial cells
	IGF 1	Regulating the proliferation and differentiation of different cell types Stimulating the production of VEGF
	bFGR	Survival/maturation of neurons and glial cells Angiogenesis
	HGF	Modulating the motility, growth and morphogenesis of various cell types Angiogenesis
	NGF	Stimulating Muller cells to produce bFGF, which then stimulates endothelial cell proliferation and secretion of VEGF
	CTGF	Stimulating proliferation, angiogenesis, migration, ECM production, cell attachment, cell survival and apoptosis
	Stem cell factor	Survival and differentiation of heamatopoetic stem cells Capillary tube formation of endothelial cells
	EPO	Anti-oxidant, anti-inflammatory, proangiogenic, neuroprotective and anti-apoptotic
	Adiponectin	Anti-inflammatory and antiatherosclerotic
Adhesion molecules	ICAM-1, VCAM-1, E-selectin	Leukocyte recruitment
	Soluble vascular adhesion protein	Leukocyte recruitment

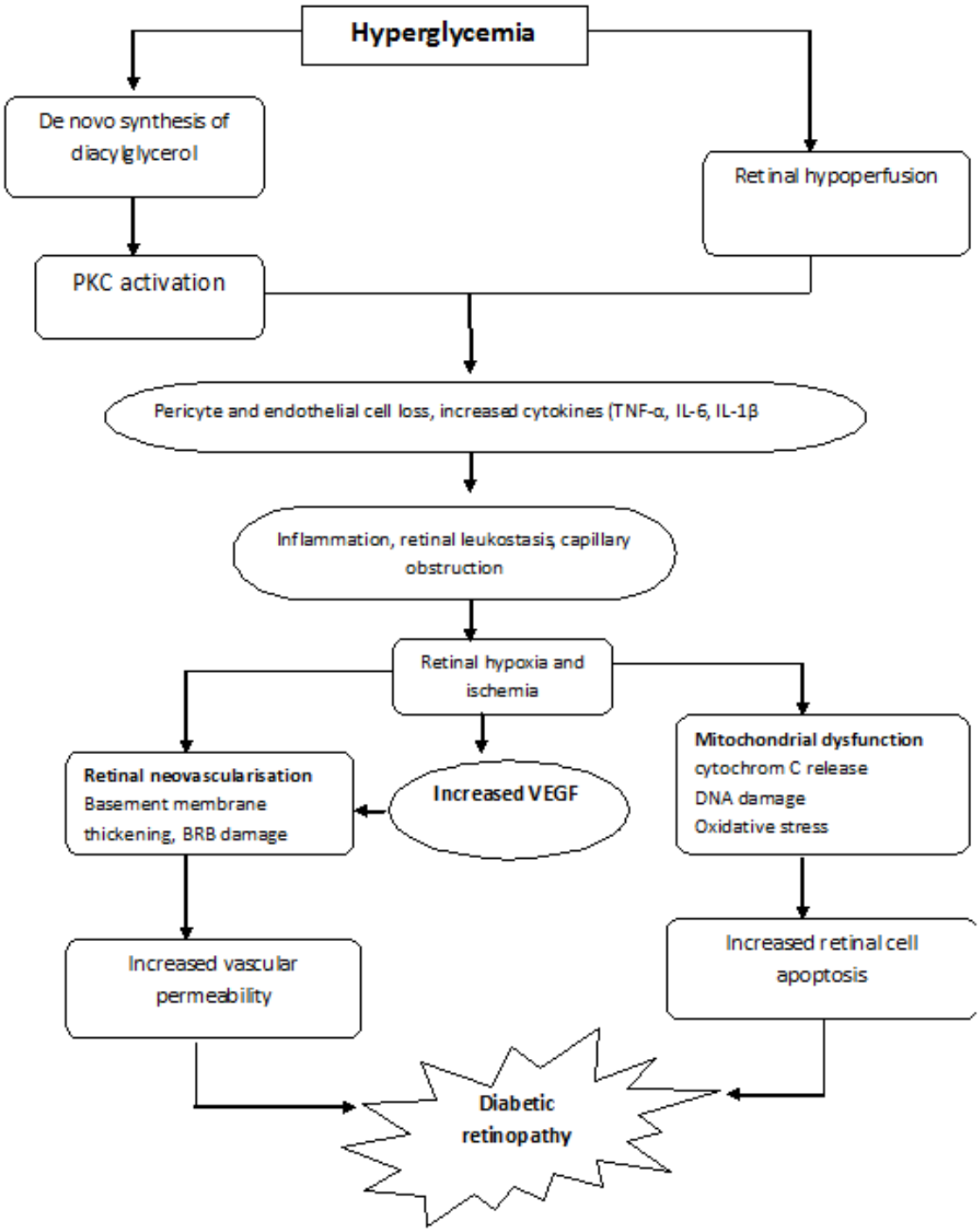
Proposed interaction of the key implicated mediators in the the pathogenesis of DR-

One of the earliest changes seen in the development of DR seems to be a decrease in retinal blood supply due to the constriction of major arteries and arterioles. (122)(124). This reduction in blood supply, results in the initiation of a cellular cascade. Among the initial inflammatory mediators, the PKC isoforms appear prominent. The PKCβII especially seems to be secreted in DR. (125). This leads to increased vascular permeability, destruction of the blood-retinal barrier and a loss in endothelial tight junctions.(122)(126). The retinal arteriolar smooth muscle cells (BK channels) dysfunction plays a key role in retinal hyperperfusion by adding to the vascular constriction.(127)(128) Additionally there is a loss of retinal pericytes. All the above eventually represent an end point of endothelial cell degeneration, destabilization and faulty perfusion of the retinal tissue.(122)(129)(130). The loss of pericytes seems to an increase in activity of OKC and a inhibition of platelet derived growth factor (PDGF).(131). Thus develops a chronic inflammation ultimately leading to capillary obstruction. (132).

This obstruction effectively results in retinal perfusion deficiencies, faulty oxygenation of retinal tissues and hypoxia. The chronic hypoxia results in secretion of a number of inflammatory mediators. The oxygen deficit with the added effect of the pro-inflammatory cytokines (TNF-α, IL-6 and -1β) leads to the enhanced expression of VEGF – now one of the emerging key players in the pathogenesis of neovascularisation of DR.

As a result of the above alterations, the retinal capillary basement membrane becomes thickened, with oversecretion of fibronectin, collagen IV and laminin, causing the formation of vessels with markedly abnormal integrity. This constitutes the stage of neovascularisation and if left untreated can progress on to advanced DR causing a loss of vision to the eye.

Figure 3. ***Outline of the pathogenesis of DR (121)***



The role of VEGF in the pathogenesis of DR

What is VEGF?

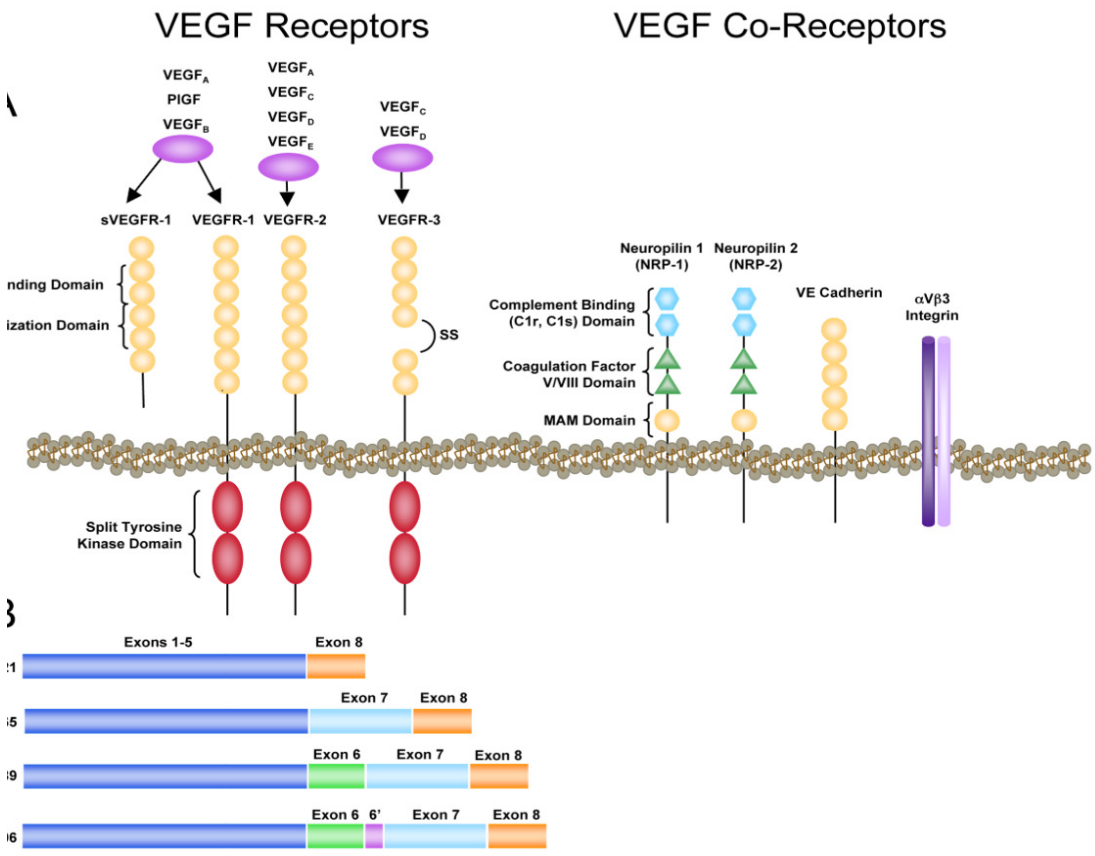
VEGF is a dimeric 40kDa glycoprotein. It is a powerful stimulator of proliferation, migration and tube generation needed for the growth of new blood vessels. It is thus essential for angiogenesis during development and a deletion of even one allele is lethal for the embryo.(133)(134)(135). There are seven members in the VEGF family: VEGF- A (commonly referred to as VEGF), VEGF-B, VEGF- C, VEGF- D, VEGF-E, VEGF-F and PIGF (placental growth factor). In addition to the above seven, splicing of VGF results in a variety of VEGF variants: such as $VEGF_{121}$, $VEGF_{189}$, $VEGF_{206}$. The degree of solubility of these variants depends primarily on their heparin binding capacity. Thus, variants which bind tightly to heparin remain mainly in the extracellular matrix (eg. $VEGF_{206}$ and $VEGF_{189}$), whereas some have no heparin binding specificity at all (VGF_{121}).

VEGF receptors-

Of the VEGF receptors (VEGFR) VEGFR-1 and VEGFR-2 are the ones mainly concerned with angiogenesis (136). The VEGFR are organized into a seven-immunoglobulin-like folded extracellular domain, leading onto a juxtamembrane part which further continues to a split tyrosine-kinase domain. This latter part has a 70-amino-acid insert and a C-terminal tail.

Dimerization is required to activate the VEGFR. Dimerization of the VEGFR causes activation of kinase activity and autophosphorylation. Tyr1214 is necessary for the autophophorylation of the receptor and thus its signaling protein activity.

Figure 4. ***The various isoforms of VEGF and basic receptor structure (137)***



Regulation of VEGF production-

Hypoxia is a very strong stimulus for VEGF expression. According to Dor et al, hypoxia causes VEGF expression by a number of mechanisms. (138). These are namely increase in transcription, mRNA stabilization, protein translation, increased oxygen regulated protein 150, which is necessary for intracellular transport of proteins. (139)(140)(141)(142).

Hypoxia inducible factor-1 (HIF-1) has a role in the increased transcription of VEGF. HIF-1 has two subunits, and inducible component HIF1-α and an innately manufactured part HIF1-β.(143) Under normal conditions, HIF1- α, is inactivated and degraded. But in hypoxia this process in inhibited and the degradation of HIF1-α is halted.(144) As a result of this, HIF1-α combines with HIF1-β, ultimately resulting in

the generation of a hypoxia responsive element (HRE). (145) Recently, HIF-2α and HIF-3α have been isolated. HIF-2α is similar to HIF-1β. However, additional studies are needed to clearly elucidate the importance of these molecules.

Evidence for the increase in VEGF mRNA comes mainly from in vitro studies. VEGF mRNA are increasingly delicate in normal conditions, during periods of hypoxia, it is protected from degradation by HuR, increasing its half life from less than an hour (in normal conditions) to about 2 to 3 hours. (146)

As mentioned earlier, there is an increase in the production of oxygen regulated protein 150 (ORP150) in hypoxic conditions. This OPR150 seems to act as a molecular chaperone to help VEGF protein transportation and secretion. Therefore, VEGF secretion is not only regulated by hypoxia. (142)

In addition, insulin-like growth factor 1 (IGF-1) also has a role in retinal neovascularisation; by affecting VEGF. (147)There are a number of studies demonstrating the importance of IGF-1 in normal angiogenesis. (148)For example, preterm babies with reduced IGF-1 have more retinal disease. Similarly mice without the IGF-1 gene have problems in retinal vasculature. (149)

<u>VEGF and VEGFR in retinal diseases-</u>

VEGF has been implicated in a number of retinal diseases. These include DR, age-related macular degeneration, retinopathy of prematurity, sickle cell retinopathy, vascular occlusions and also in neovascular glaucoma.(150)

There seem to be 5 cells of the retina capable of secreting VEGF. These are the(151)(152)(153)

- Retinal pigment epithelial cells (RPE)

- Astrocytes
- Muller cells
- Vascular endothelium
- Ganglion cells

However in conditions of hypoxia, studies show that Muller cells and astrocytes produce the most amounts of VEGF. (154)The exact role of the splice variants is still unclear. However it is seen that VEGF $_{120/120}$ mice retinas had severe vascular pathology, $VEGF_{188/188}$ had normal retinal veins but no arteries.

In human adults, expression of VEGF is restricted to the inner nuclear layer (ei. the Muller cells and the amacrine cells), the ganglion cells and the retinal vessels.(155) During neural development of the retina, VEGFR-2 can also be manufactured by the neural progenitor cells.(156) Interestingly, VEGFR-1 and 2 has not been found to be expressed by retinal smooth muscles.

VEGF and DR-

That there is an association between VEGF and the pathogenesis of DR is now an undisputable fact- especially, considering the number of studies showing a positive correlation between increased intraocular levels of VEGF and PDR. Animal studies by Gilbert et al have demonstrated retinal changes similar to background diabetic retinopathy by increasing VEGF levels and increasing VGEFR2 expression.(157)(158) Blocking VEGF activity has been shown to prevent the development of diabetes-induced vascular permeability, thereby underlining the importance of VEGF in the pathology of diabetic macular edema (DME) by increasing vascular permeability.(159)(160)(161)

VEGF gene expression in DR-

VEGF expression-

The regulation of the VEGF gene is very tightly controlled and over-expression has been linked to a number of diseases; including DR. The extent of activity of VEGF is positively linked to the expression of its gene. In diabetes, many factors act towards the over-expression of VEGF, such as tissue hypoxia, growth factors, inflammatory cytokines and reactive oxygen radicals. In the next few paragraphs we will go through the regulatory mechanisms for VEGF gene expression and how they are important in diabetes

Transcriptional regulation of VEGF gene expression

Hypoxia is a most important factor and the most studied one for VEGF regulation. Studying diabetic mice, de Gooyer et al reported a decrease in retinal capillary density along with periods of hypoxia.(162) As already stated, the HIF-1 transcription factor is involved in this transcriptional increase in VEGF. However, these increases in VEGF levels are detected very early on, before any capillary drop out has been detected. Therefore the exact mechanism behind the hypoxia induced over-expression of VEGF is still not clearly known.

Apart from hypoxia, TGF-β, TNF-α. IGF-1, advanced glycation products and oxidative stresses are involved in the upregulation of VEGF expression in diabetes. (163)(164)(165)(166). The exact mechanisms by which these factors achieve such an end point is not clear and seems to be complicated, to say the least.

The post-transcriptional regulation of VEGF expression-

Though the VEGF post-transcriptional events (in normal and abnormal situations) is known, how elevated sugars alter the post-transcriptional events is not so well known. There seems to be a change in mRNA stabilization and activation of multiple internal ribosomal entry sites(IRES) . IRES is involved in the production variant VEGF isoforms splices. How this plays a role in DR will be explained in the next section.

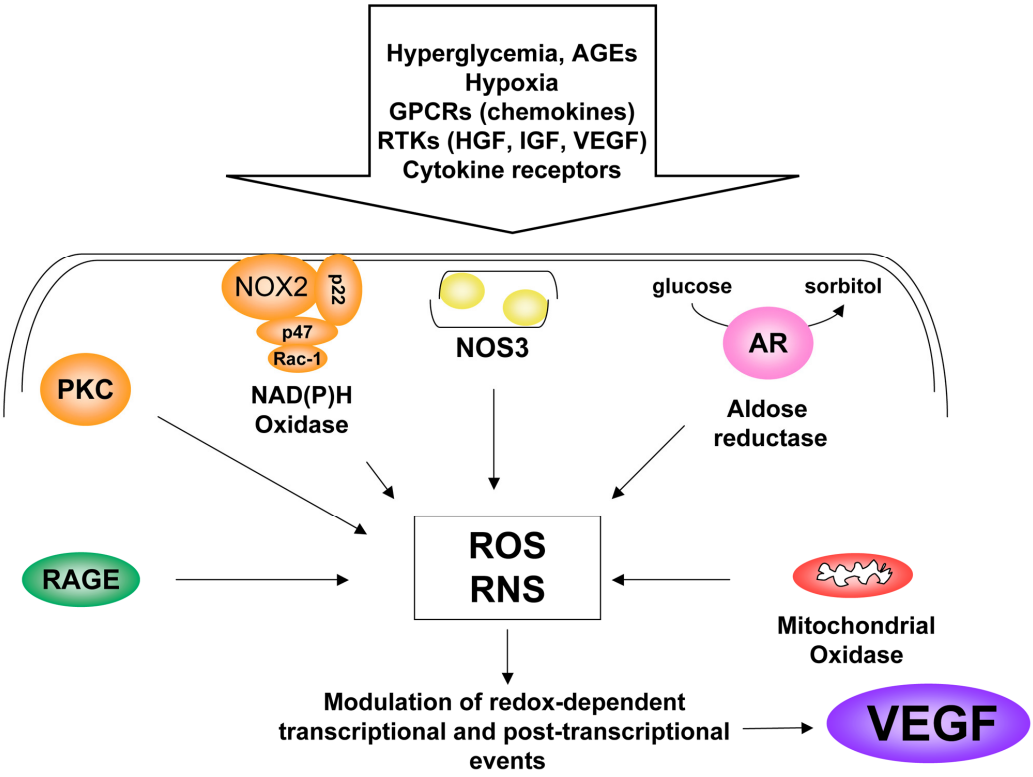
VEGF splice variants-

The different splice variants of VEGF play a number of parts in retinopathy. VEGF165 seems to be a powerful agent of retinal inflammation and also neuronal survival. (167) (168)In contrast VEGF121 is involved only in neuronal activity and not in inflammation. In vitreous from eyes with DR, there was found to be decreased levels of the negative splice variant VEGF165b, pointing to a molecular switch between VEGF165 and VEGF165b. (169)

VEGF autocrine activity in the endothelial cells-

Though VEGF is mainly a paracrine factor, in hypoxia, it appears to have autocrine abilities- stimulating its own production form endothelium of the microvasculature.(170). The exact importance of this autocrine secretion is not known, but high glucose and reactive oxygen species can induce VEGF expression for retinal endothelial cells in experimental conditions. Possibly these inhibit proteins that are normally inhibitory to the production of VEGF.

Figure 5. ***VEGF gene upregulation leads to increase in VEGF expression and activity(137)***



VEGF activity regulation in DR

VEGF and Vascular inflammation-

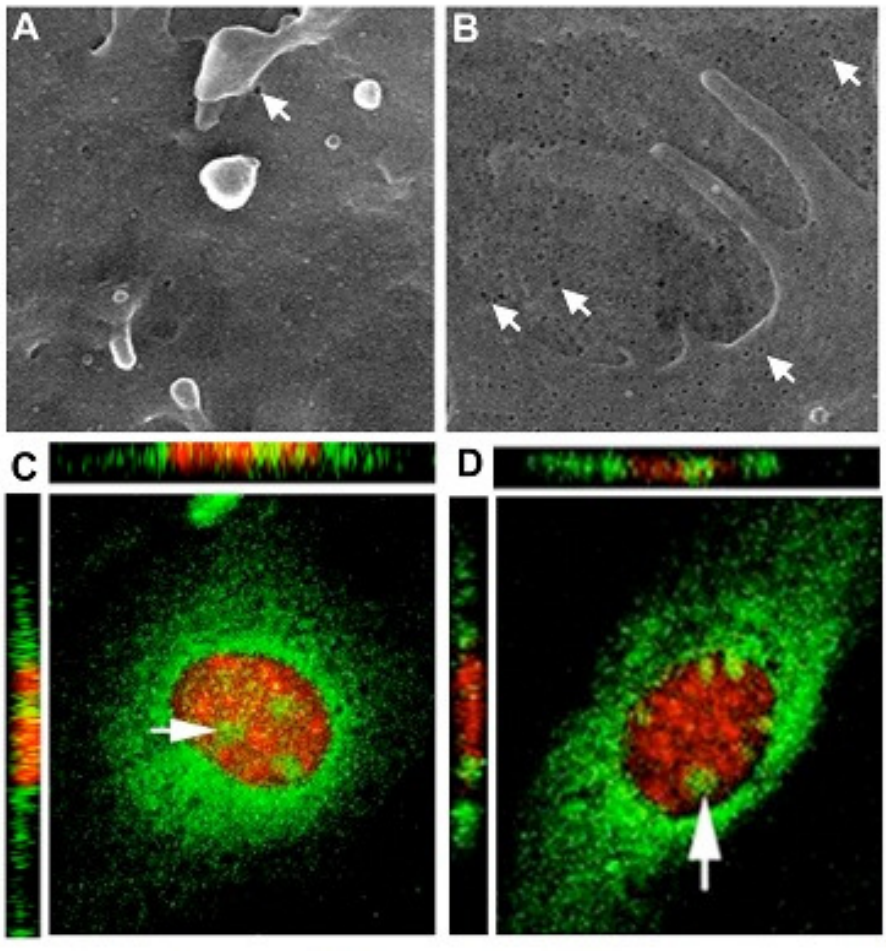
A number of facts have been experimentally demonstrated to shown that VEGF has a role in inflammation and DR. Endothelial cells treated with VEGF show increased expression of ICAM-1 and MCP-1. (171)(172)(173)The retinas of diabetic animals show increases in VEGF expression correlating with increased ICAM-1 immunoreactivity and leukostasis. Specifically VEGF165, as mentioned earlier has a role in this and intravitreal injecton has been shown to induce the expression of ICAM-1 in retinal vasculature.

VEGF and Vascular permeability-

There is a breakdown in the blood-retinal barrier of the vascular endothelium in diabetes. This breakdown correlates with increased VEGF in ocular specimens.(174)(175)(176) There are a number of pathways implicated in this increase in vascular permeability.

As demonstrated by Roberts et al, topical or intradermal application of VEGF is followed by an increase in capillary permeability, formation of fenestrations and a trans-cellular permeability pathway.(177). Further studies have shown that VEGF treatment results in an initial rapid increase in transcellular permeability lasting about an hour. This is facilitated by a transcytotic transport of caveolin-coated vescicles and was accompanied by nuclear translocation of VEGFR2. This was then followed by a more sustained increase in vascular permeability which involved the translocation of β-catenin. (178)

Figure 6. ***Experimental model showing VEGF induced increase in caveoli***

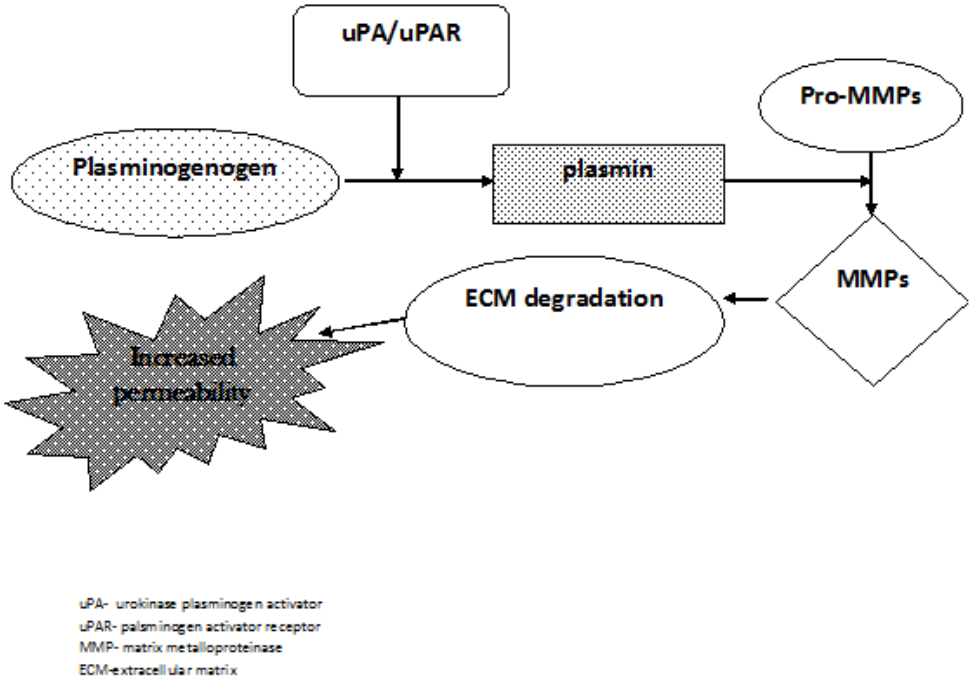


(137)

A- Very few caveolae in the normal retinal endothelial cell (white arrow)
B- VEGF treatment induces a significant increase in caveolae
C- Nuclear translocation of VGFR2 due to VEGF treatment (green label)
D- Nuclear translocation of NOS3 due to VEGF treatment (green label)

VEGF-induced increase in vascular permeability probably occurs as a result of endothelial cell-to-cell attachment disruption involving interactions between MMP-9, plasmin and uPAR on the cell surface. (179)(180)(181)This proteolysis alters attachments between cells, thus generating leaky vessels, allowing endothelial cell penetration of basement membrane. After this the cells can migrate and proliferate unchecked, setting the scene for retinal neovascularisation. This schema of events has been supported by a number of experimental studies.

Figure 7. ***Postulated interactions for the increased permeability of DR (137)***



Retinal angiogenesis-

Vasculogenesis is a term used to describe a phenomenon where marrow-derived endothelial progenitor cells (EPC) in circulation are recruited and incorporated into new vessels. VEGF, apart from it's well know role in angiogenesis, has a part to play in altering vasculogenesis. (182) (183)(184) EPC's from diabetic patients have impaired proliferation, adhesion and incorporation into the blood vessels.(185) Studies in an experimental environment of retinal ischemia have demonstrated the participation of marrow derived hematopoetic stem cells in neovascularistion.(186) Furthermore, normal EPCs can in fact repair injured retina. EPCs from diabetics have been demonstrated to be unable to perform this function effectively. (187)

VEGF and cell longevity-

VEGF has a definite contribution to endothelial cell survival. Ironically, in the diabetic, though levels of VEGF and VEGFR2 are increased, the endothelial cell survival is reduced. (188)(189) This fact is well-established by the presence of acellular capillary capillary network and increased apoptosis of the endothelial cell. These seemingly contradictory discoveries suggest some additional alteration to the VEGF mediated survival of endothelial cells in the diabetic environment.

Analysis of VEGF

Ocular fluid-

In the original study by Aiello et al, VEGF concentrations in ocular samples were measured by a radioimmunoassay using two monoclonal antibodies bound to different epitopes on VEGF 165 and other larger isoforms. They performed the assays using 96-well immunoplates. Each of these plates were coated with 100µl of anti-VEGF monoclonal antibody. The plates were then washed thrice, blocked for one hour at 25 degree Celsius with 0.5% bovine serum albumin and 0.03 percent Tween 80 in phosphate-buffered saline. They are then washed with 0.03 Tween 80 before adding diluted sample of ocular fluid or standard solution of VEGF165.

The plates were then kept for a 2 hour incubation at 25 degree. After discarding the supernatant fluid, the wells were washed and 100 microlitres of ^{125}I-labeled anti-VEGF monoclonal antibody was added. The plates again underwent a two hour incubation. At 25 degree, after discarding the supernatant, and washing, the wells were subjected to counting by gamma scintigraphy. The VEGF concentrations were quantified from a standard VEGF curve.

Recent studies now use enzyme linked immunosorbent assay (ELISA) kits to measure VEGF concentrations.

Blood- serum or plasma-

For the analysis of VEGF levels in serum and plasma samples, ELSIA kits are commonly used.

In our study we have used the Neogen Corporation's Human VEGF ELISA Test kit. This is designed for the quantitative determination of human VEGF in serum, plasma and cell cultures.

Briefly, the procedure involves a solid phase sandwich ELSIA technique. A monoclonal antibody specific for human VEGF is coated onto 96 wells. Samples are added to the wells and any human VEGF present binds to the antibody-capture antibody.

The wells are then washed and biotinylated polyclonal anti-human VEGF antibody (detection antibody) is added. After washing again, avidin-horseradich peroxidase (avidin-HRP) is added, thereby resulting in an antibody-antigen-antibody sandwich. The wells are washed again and substrate solution added. This is to produce a blue color in proportion to the amount of human VEGF in the test (initial) sample.

This kit has a sensitivity of 15pg/mL and a specificity of 100% for human VEGF.

Study of VEGF levels in DR

Ocular fluid and VEGF in DR

The search for a "hypothetical angiogenic substance"-the culprit for the devastating complications of neovascularisation seen in DR ended in 1994 when Aiello et al in their paper analysed VEGF levels from the ocular fluid of patients with pathological neovascularisation. They reported VEGF detection in 69 of the 136 samples from patients with DR, 29 of 38 samples from patients with iris neovascularisation, and 3 of 4 sample of patients with vein occlusions as compared to 2 out of 31 patients with no vascularisation.(4).

Figure 8. ***VEGF levels from ocular fluids Aiello et al (4)***

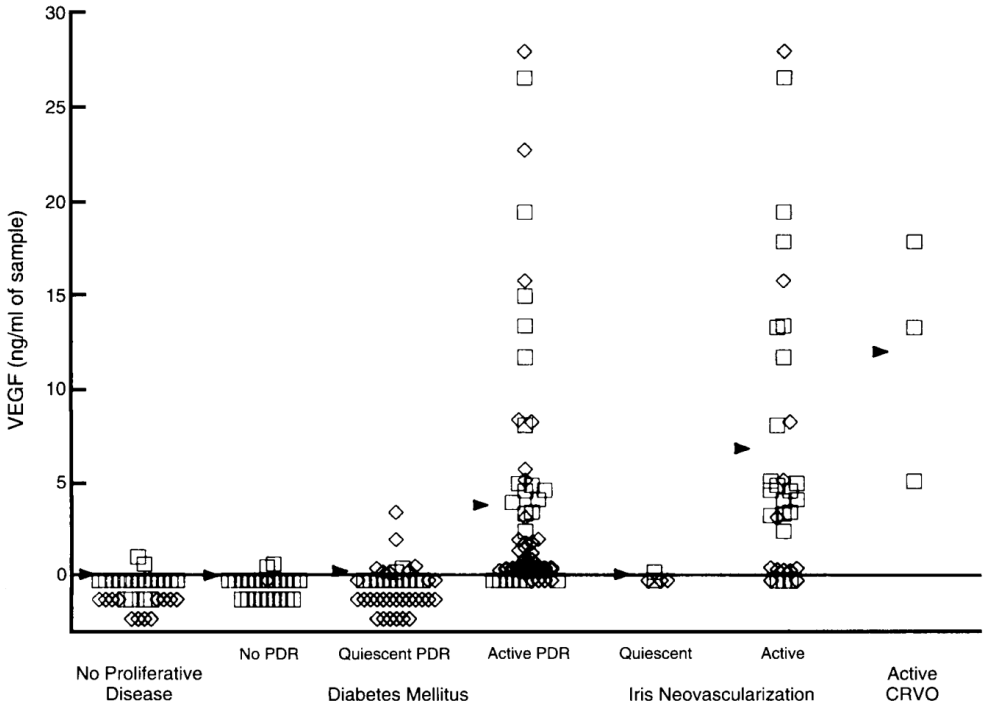


Figure 2. Concentrations of Immunoreactive VEGF in Ocular Fluids from Patients Undergoing Intraocular Surgery.

As can be seen from the above graph, VEGF was significantly elevated in patients with active neovascularisation.

Subsequently, there have been numerous reports of increased VEGF concentrations in vitreous, aqueous, neovascular membranes and vitreous hemorrhage of patients with DR.

Serum and plasma levels of VEGF in DR-

The literature on serum levels of VEGF and DR can at best, be described as, contradictory.

There is one study by Hellgren et al where they report an increase in levels of circulating VEGF in preterm infants who later went onto develop severe retinopathy of prematurity. (190).

Meleth et al in a study on serum inflammatory markers in DR report a significant elevation in the chemokines RANTES and SDF-1apha. There is no clear data regarding VEGF levels in this study.(191)

A study by Semeraro et all where they compared systemic and intraocular concentrations of VEGF and erythropoietin (EPO) between 33 type 2 diabetics and undergoing vitrectomy for PDR patients (cases) with 20 patients undergoing vitrectomy for macular holes or puckers (controls).(192) They reported no significant difference in serum levels of EPO between the two groups. VEGF levels in serum were paradoxically lower in diabetics. The authors conclude that there must be other confounders affecting the serum concentrations of these markers. This has also been suggested by GUo et al where they report a positive association between serum EVGF, ferritin and the development of retinopathy. (193)

Another study by Fan et al on 1040 Chinese people with type 2 diabetes reported higher serum VEGF levels in patients with retinopathy than those without. (194) Koleva-Gerogieva et al also report similar findings in their study on serum inflammatory cytokines and DR. (195) Li et al also state the importance of serum cytokines and VEGF in the diagnosis of PDR in their study on 30 patients. (196)

However, Praidou et al report no such correlation between serum levels of VEGF and DR.(197)

Soiberman, Davidovic et al, on the other hand, in their report on serum levels of VEGF following intravitreal ani-VEGF injections describe a decrease in serum VEGF level following the injections. (198) (199).

The studies are many and a variety of associations between serum VEGF and DR have been proposed and disputed. There is one study by Cavusoqlu et al on 65 patients studying the serum levels of VEGF at different stages of DR. However they state that the levels were different in the groups. They report a correlation between serum VEGF and HbA1c levels.(200) To the best of our knowledge there is no report thus far on serum VEGF, grading of diabetic retinopathy in such a large number.

MATERIALS AND METHODS

Materials and Methods

Study population-

Outpatients presenting to the Ophthalmology department of PSG Hospitals with clinical diagnosis of Type 2 diabetes mellitus for routine diabetic retinopathy screening.

Inclusion criteria-

1. Type 2 Diabetics with retinopathy

2. Type 2 diabetics without retinopathy

Exclusion criteria-

1. Type 1 Diabetes Mellitus

2. Other retinal diseases

3. Terminally ill patients

4. Patients who have received prior anti-VEGF therapy.

Sample size-

Due to money and time constraint a convenient sample of 75 patients with type 2 diabetes and presenting to the outpatient department for routine diabetic retinopathy screening were chosen. This sample size was chosen after reviewing the existing statistics collected from the department register which showed that on an average 30-32 patients attended our OPD over a period 3 months for diabetic eye screening.

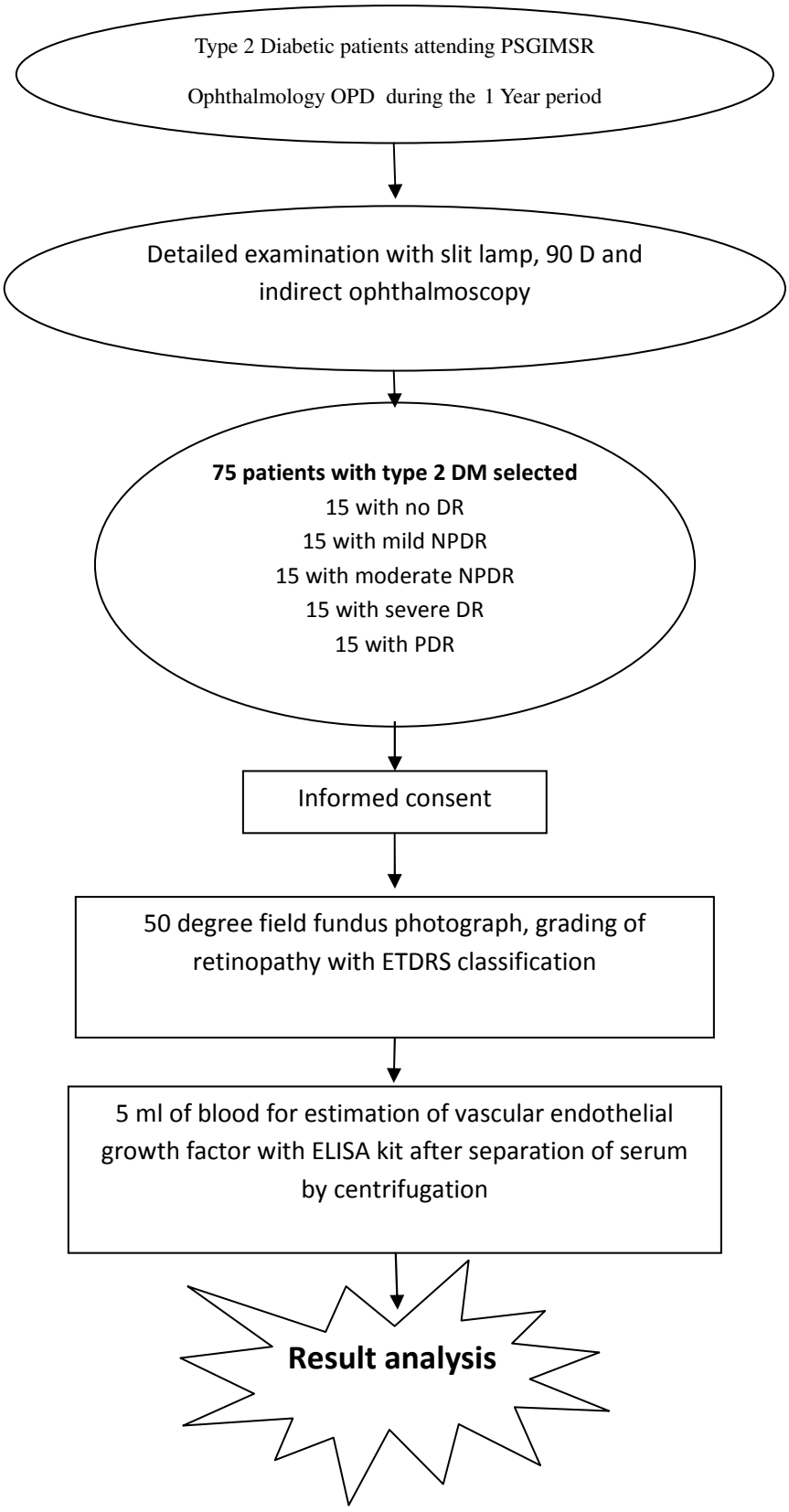
Tool used-

- ETDRS classification of diabetic retinopathy based on 50^0 7 field fundus photography
- Human VEGF ELISA Kit

Study design:

Descriptive analytical study

Methodology-



ELISA-

The concentration of serum VEGF levels in the sample will be quantitatively measured by using ELISA kit for human VEGF(Neogen U.S.A Human VEGF Product #452610)

Statistical analysis-

Analytical software used was SPSS version 19.

Count data were analyzed using the Chi-square test. Continuous variables were analyzed using the Students's *t* test, Independent one sample *t* test, and Analysis of variance (ANOVA).

RESULTS

Results

Demographic characteristics of the population under study.

Total number of patients- 75

No NPDR=15

Mild NPDR=15

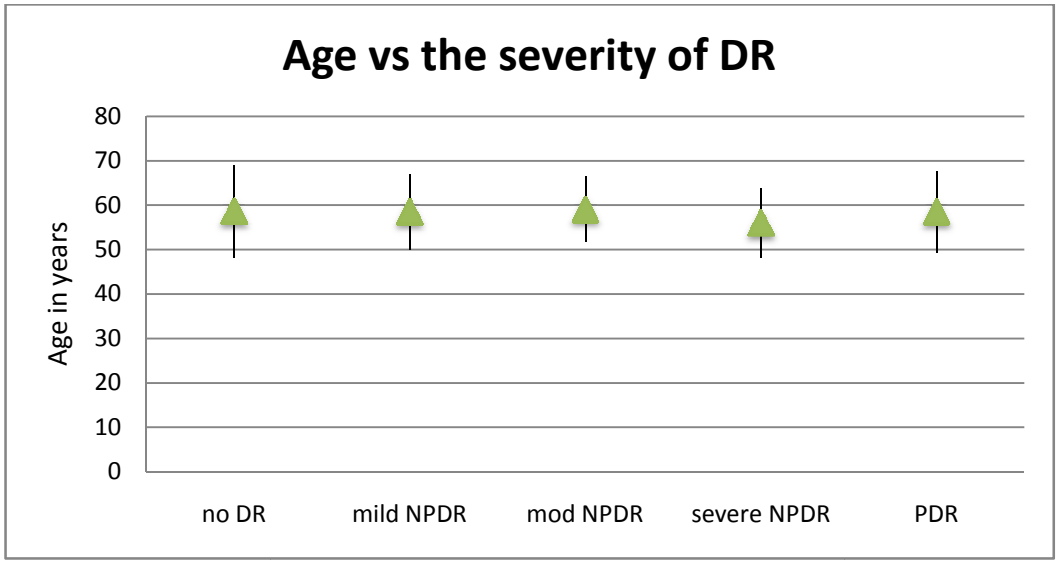
Moderate NPDR=15

Severe NPDR=15

PDR=15

	Average in study population	No DR	Mild NPDR	Moderate NPDR	Severe NPDR	PDR	*P* value
Age	58.16 years (SD=8.6)	58.67 years (SD=10.4)	58.53 years (SD=8.4)	59.07 years (SD=7.3)	56.00 years (SD=7.8)	58.53 years (SD=9.1)	0.880
Sex ratio (M:F)	2.75:1	1.5:1	2.7:1	2.7:1	6.5:1	2.7:1	

P value less than 0.05 taken as significant.



P value= 0.88

Primary outcome measures

VEGF levels

VEGF and the normal population

Overall mean VEGF levels for the study population of 75 diabetic patients was 577.01 pg/ml (SD= 291.13)

On reviewing the literature, the normal limits for VEGF were found to be 72-707 pg/ml. Assuming that the range of VEGF for the normal population follows a typical bell-shaped distribution, we used the independent one sample t-test to compare our mean VEGF result with the normal range. Our VEGF levels were found to be very significantly elevated when compared with the normal population. (p value =0.00001.)

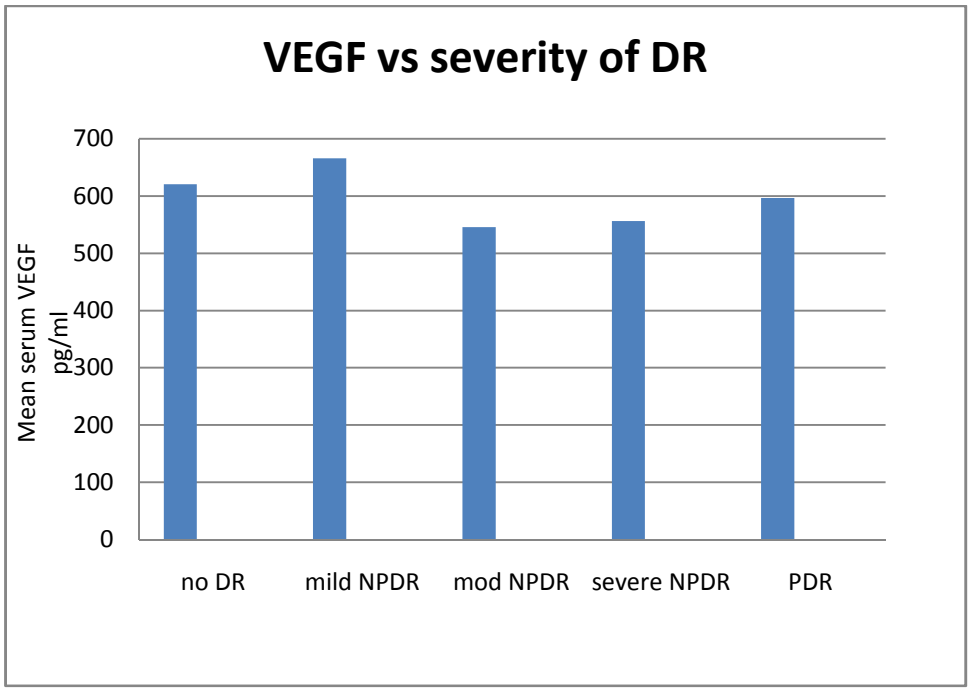
Comparing specifically, the VEGF in patients with no DR and the normal population was also significantly elevated. (p value= 0.0076)

VEGF levels and the grade of DR

The lowest value of VEGF was seen in the no DR group (34 pg/ml). The highest value was seen in the mild DR group (1296 pg/ml).

Overall, there was no statistical significance in the variation of VEGF and the grade of DR. (p value=0.544).

	Mean VEGF (pg/ml)	Standard deviation
No DR	620.73	293.50
Mild NPDR	666.15	314.68
Moderate NPDR	545.06	264.13
Severe NPDR	556.06	293.53
PDR	496.48	296.43



P value=0.544
(p value less than 0.05 taken as significant)

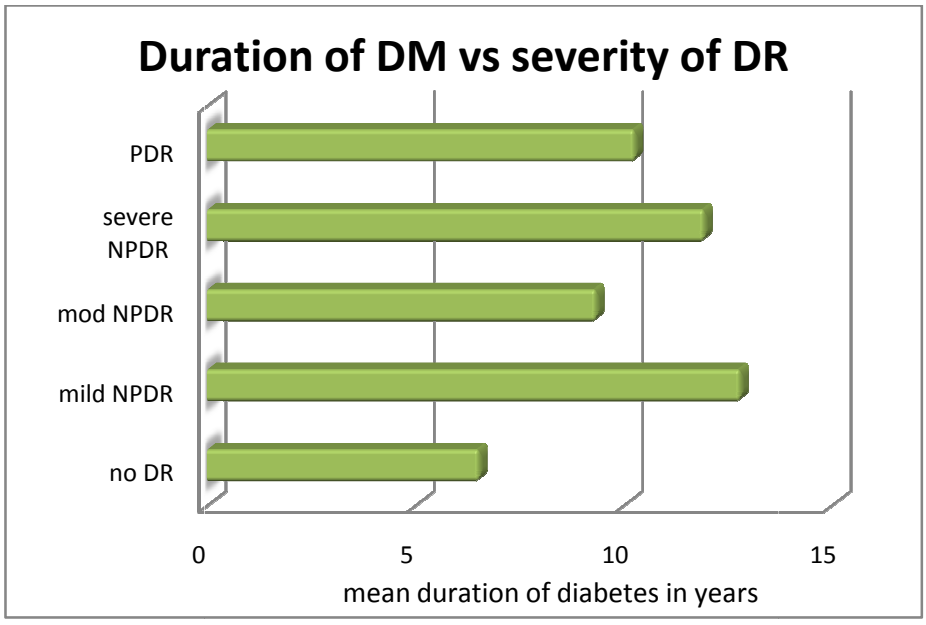
Secondary outcome measures

Duration of diabetes

The average duration of diabetes in the study population was 7.2 years.

The subpopulation without any diabetic retinopathy had the minimum duration of 6.53 years. The population with mild NPDR had the maximum duration of 12.80 years. There was no statistically significant difference in the duration of diabetes and the grade of diabetes. (p value=0.137)

	Duration of diabetes in years	Standard deviation
No DR	6.53	6.73
Mild NPDR	12.80	9.38
Moderate NPDR	9.33	5.62
Severe NPDR	11.93	6.66
PDR	10.27	6.23



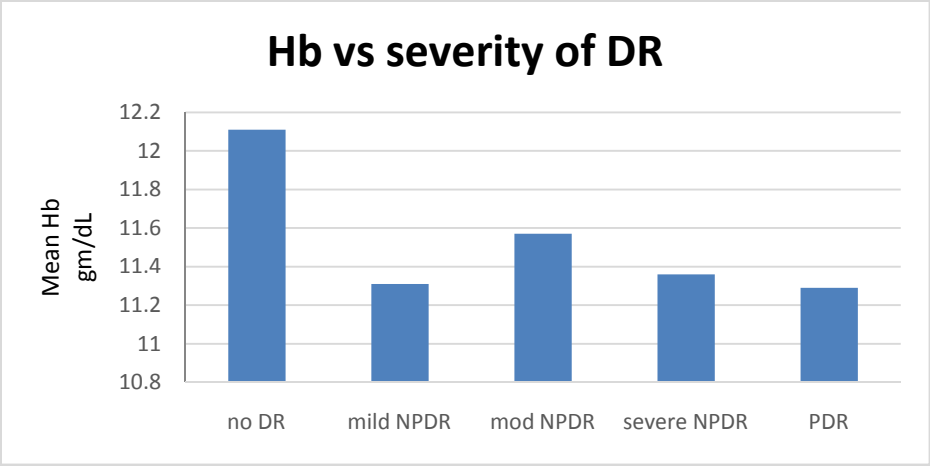
P value=0.137
(p value less than 0.05 taken as significant)

Heamoglobin levels

The average haemoglobin level in the study population was 11.53 gm/dl (SD=2.00).

There was no significant difference in the haemoglobin levels and the severity of diabetes. (p value=0.791)

	Mean haemoglobin in gm/dl	Standard deviation
No DR	12.11	1.86
Mild NPDR	11.31	2.32
Moderate NPDR	11.57	2.61
Severe NPDR	11.36	1.69
PDR	11.29	1.47



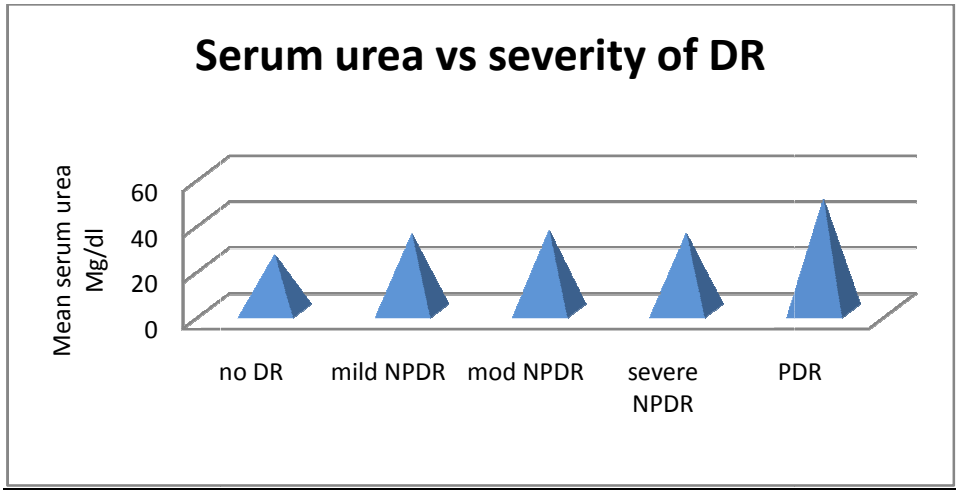
P value=0.791
(p value less than 0.05 taken as significant)

Urea levels-

The mean urea levels in mg/dl of the whole population was 35.47 mg/dl (SD=25.80).

There was no significant difference in the levels of urea and the severity of diabetes (p value=0.138).

	Mean urea levels in mg/dl	Standard deviation
No DR	24.73	19.36
Mild DR	33.90	17.95
Moderate DR	35.45	28.97
Severe DR	34.10	29.43
PDR	49.17	28.07



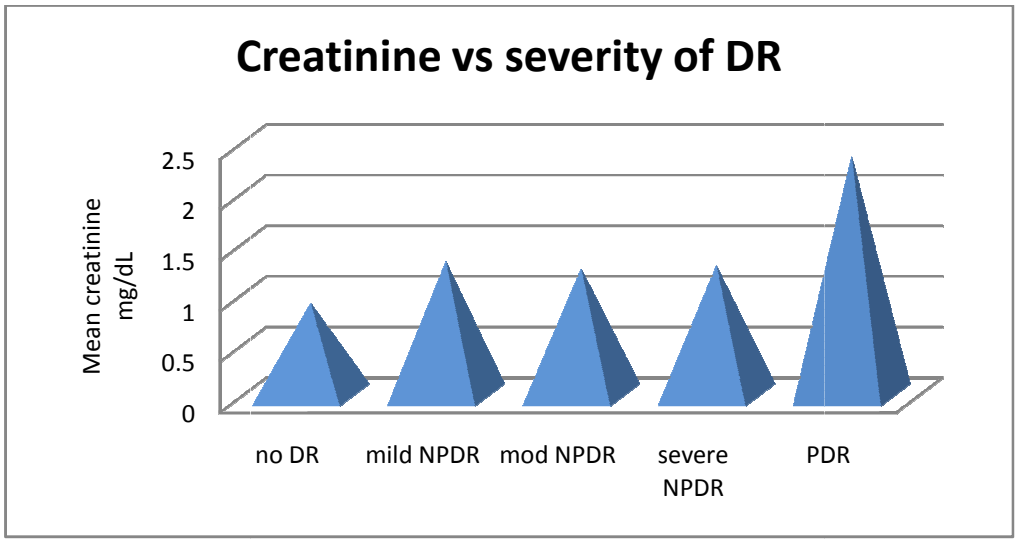
P value=0.138
(p value less than 0.05 taken as significant)

Serum creatinine levels

The mean creatinine levels for the population was 1.42mg/dL (SD=1.55).

The minimum value was seen in the patients with no DR (0.91 mg/dL). The highest values were seen in the group with PDR (2.26 mg/dL). However any intergroup variation of creatinine was not statistically significant. (p value= 0.11)

	Mean creatinine values (mg/dL)	Standard deviation
No DR	0.91	0.76
Mild NPDR	1.32	1.03
Moderate NPDR	1.25	1.09
Severe NPDR	1.28	1.16
PDR	2.36	2.69



P value= 0.11 *(p value less than 0.05 taken as significant)*

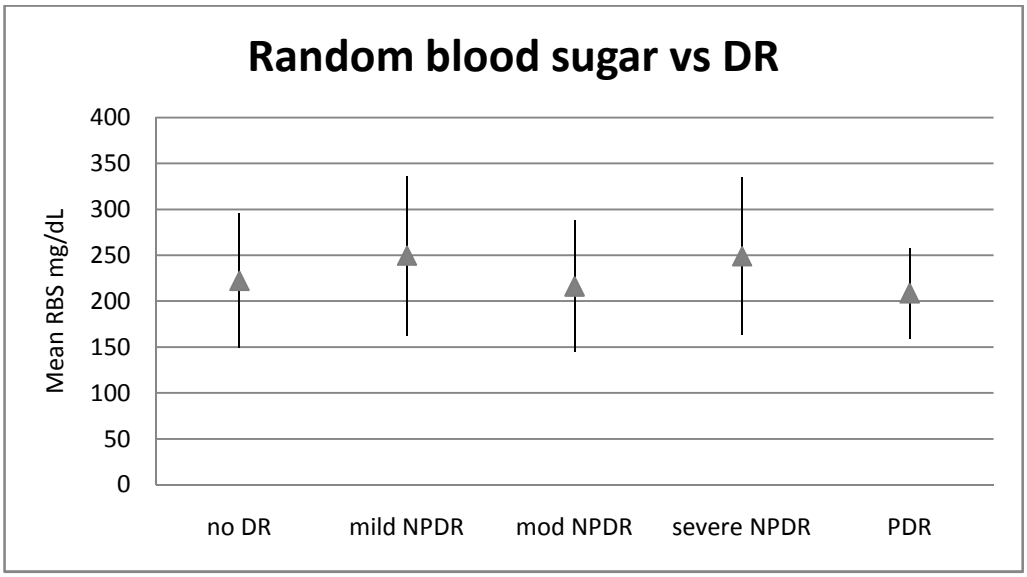
Random blood sugar levels

The mean random blood sugar levels for the whole population were 228.95 mg/dL (SD=74.499).

The normal random blood sugars were taken to be 70-140 mg/dL. After statistical analysis, the raise in the mean random blood sugar was found to be significant.

However, there was no statistically significant change in the random blood sugar levels and the severity of diabetes (p value=0.430).

	Mean random blood sugar mg/dL	Standard deviation
No DR	222.07	73.222
Mild NPDR	249.07	86.633
Moderate NPDR	216.00	71.762
Severe NPDR	249.00	85.675
PDR	208.60	49.406



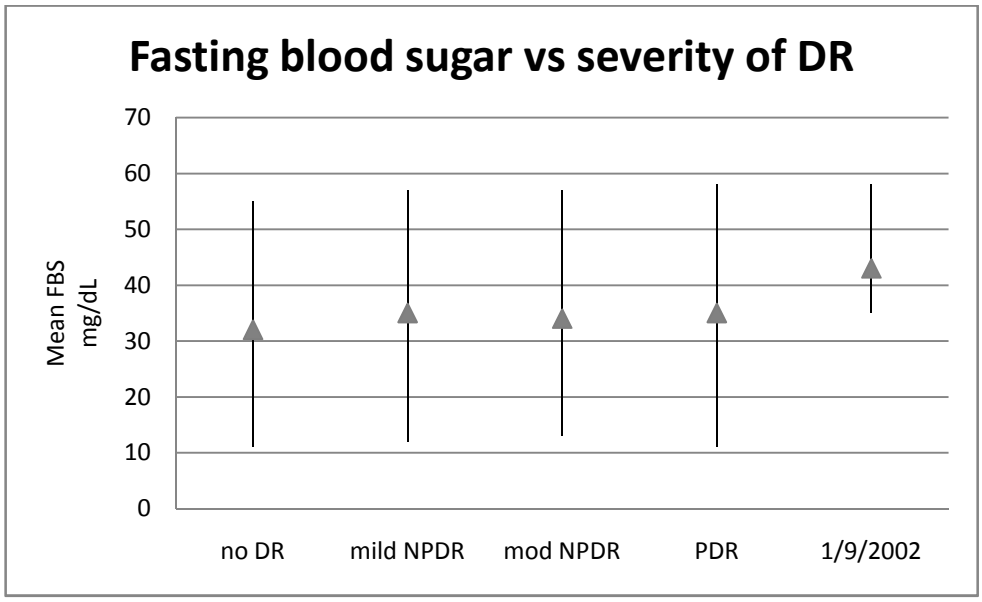
P value=0.430
(p value less than 0.05 taken as significant)

Fasting blood sugar levels

The mean fasting blood sugar levels for the 75 patients was 165.87 mg/dl (SD=59.04).

However, there was no significant difference in the fasting blood sugar levels and the severity of diabetes (p value=0.408)

	Mean fasting sugars mg/dL	Standard deviation
No DR	162.87	55.564
Mild NPDR	183.00	82.532
Moderate NPDR	156.67	40.334
Severe NPDR	179.13	54.536
PDR	145.67	54.052



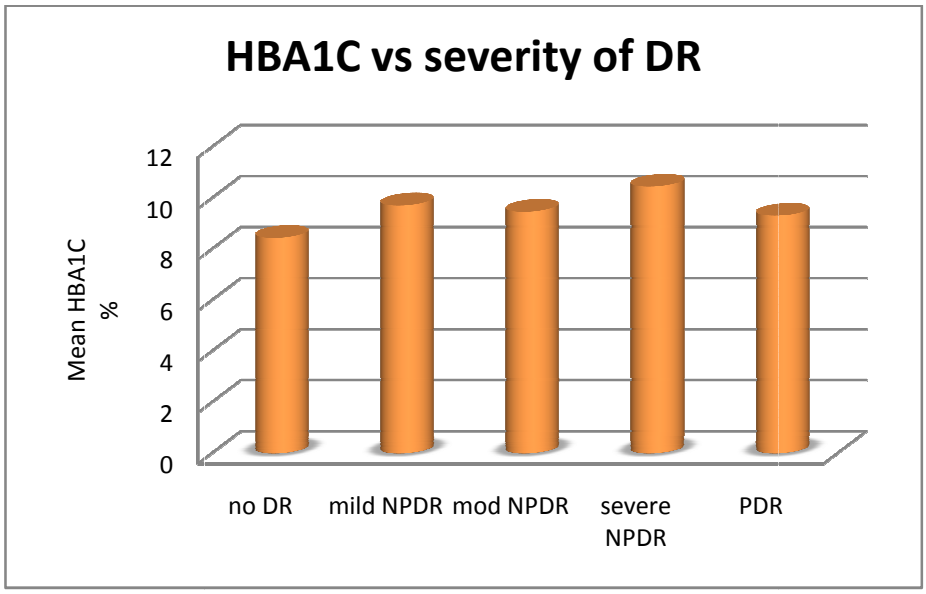
P value=0.406
(p value less than 0.05 taken as significant)

HBA1C levels

The mean HBA1C levels were 9.49% (SD=2.37)

The lowest HBA1C levels were seen in the group with no DR (8.45%) and the highest was seen with the group with severe NPDR (10.46%). However, the difference was not statistically different (p value- 0.230)

	Mean HBA1C (%)	Standard deviation
NO DR	8.45	1.57
Mild NPDR	9.72	2.06
Moderate NPDR	9.47	1.65
Severe NPDR	10.46	3.45
PDR	9.32	1.90



P value=0.230
(p value less than 0.05 taken as significant)

Discussion

Diabetic retinopathy is without doubt one of the most significant ocular complications of a systemic disease. Unfortunately the numbers of patients with DR is on the rise and it is one of the most common causes of blindness in many parts of the world, especially of the middle aged population. It has been shown in a countless number of studies that the vasculogenic factor –VEGF- plays a prime role in the pathogenesis of the DR. That vitreous levels of VEGF are elevated in patients with DR- in particular active, advanced stages of DR- is an undisputable fact. However, the association of blood VEGF levels and diabetic retinopathy has not been studied quite so much, and whatever studies are there report conflicting data.

The aim of our study was to study serum VEGF levels of diabetic patients with and without diabetic retinopathy, and if there was any such association, to discern a relationship of serum VEGF levels with the severity of diabetic retinopathy.

Our study design was a descriptive analytical study of patients with diabetes mellitus type 2 presenting to our out patient department for routine diabetic screening. . We selected a representative sample size of 75 patients, which included 15 patients without any retinopathy, 15 with mild DR, 15 with moderate DR, 15 with severe DR and 15 with PDR. After obtaining informed consent from the patient, we drew a 5 ml sample of venous blood for the analysis. The sample was subsequently subjected to centrifugation and using the human serum VEGF ELISA kit (Neogen U.S.A Human VEGF Product #452610) the VEGF levels in the serum sample were obtained.

Demography-

The average age of the patients in the study population was 58.16 years. This result is to be expected as type 2 diabetes mellitus is mainly a disease of the middle aged and our study was only on type 2 diabetics. Any patient with type 1 diabetes was excluded from the study.

Earlier studies done in similar populations from South India report age and longer duration of diabetes as risk factors for DR. (201)(202)(73). However, in our study, further analysis of the age and the severity of diabetes showed no statistical significance.

Regarding the association with gender, the majority of the patients were male, with an overall male to female ratio of 2.75:1. A similar result was reported by Raman et al, where they report an increased risk of DR among male patients (Odds ratio=1.41). (202).

Primary outcome measures-

The serum VEGF level in the 75 patients was taken as our primary outcome. The average serum VEGF levels were 577.01 g/ml. On comparing this value with the known normal serum VEGF levels, our result was proven to be very significant. This result has been reported in a number of other studies as well. (194)(195).

We then analyzed the elevation between the group with no DR and the normal population values. This too was significantly elevated (p value= 0.0076). Therefore, it appears that VEGF is elevated in diabetics per se, and not specifically in patients with diabetic retinopathy. This can be explained by the fact that VEGF is a systemic factor and not confined to the eye. VEGF has been implicated in the pathogenesis of not only

diabetic retinopathy, but also in diabetic nephropathy, neuropathy and even diabetic macrovascular disease.(203)(204)(205)

Paradoxically, the group with PDR had the minimum mean value of VEGF. The group with mild NPDR had the maximum mean VEGF value. On testing the relation between VEGF and the severity of DR, there was no statistical significance detected. Similar results have been obtained in a number of other studies. (197)

Hence, to answer our primary research question, serum VEGF levels are elevated in diabetics when compared with the normal population. But there is no relationship with the severity of diabetic retinopathy.

Secondary outcome measures

Duration of diabetes-

In our study, the average duration of diabetes was 7.2 years.

There was no statistically significant relationship between the duration of diabetes and the severity of diabetes. However, the group with the minimum number of years of diabetes was the group with no DR. This is in keeping with the established fact that the duration of diabetes is an important risk factor for the development of retinopathy.

Heamoglobin level-

The mean heamoglobin level was 11.53gm/dL. Taking the normal range of heamoglobin to be 12-15gm/dL, our population appears to be on the aneamic side. In addition, the group with PDR had the lowest mean heamoglobin of 11.29; the group with the highest heamoglobin was the group with no DR.

Though this is not statistically significant, this trend is in keeping with the fact that anemia worsens the retinopathy status. Additionally, treating anemia has been proven to improve retinopathy and delay its progression. (96).

Urea and creatinine levels-

The average urea level for our population was 35.47 mg/dL. Taking the normal range of serum urea to be from 14-40 mg/dL, this value falls into the high normal range.

The mean creatinine levels were 1.42 mg/dL. As the normal range of creatinine is 0.66- 1.09 mg/dL, this definitely falls into the high range.

There was no statistically significant relationship between the creatinine levels or urea levels and the severity of diabetes. Even so, the group with PDR had the highest urea levels and creatinine levels. Likewise, the group with PDR had the highest urea and creatinine levels.

This is very much in keeping with the fact that both nephropathy and retinopathy are microvascular disease and often the presence of one indicates the presence of the other. However, there is also a good chance of this renal disease being non-diabetic in origin. As highlighted in an earlier study by Prakash et al, nondiabetic renal disease versus diabetic disease was 22.6% vs 3.2% when the duration of diabetes was between 5 to 10 years. It is only after 10 years of diabetes that, diabetic renal disease jumped ahead to 32.2% vs 6.5% for non diabetic renal disease. (206). As our population mean duration of diabetes was 7.2 years, it is likely that the any renal impairment is more of a nondiabetic origin.

Random blood sugar and fasting blood sugar levels-

The average random blood sugar levels for the whole population was 228.95 mg/dL and the mean fasting blood sugar levels was 165.87 mg/dL. Both these values are elevated when compared with normal values. This fact not only highlights the chronic hyperglycemia associated with diabetes, but also that most of patients seem to have suboptimal sugar control.

This fact has important long-term prognostic value as it has been proved in numerous studies that control of blood sugar and other co-morbidities play a major role in the control of diabetic complications.

There was no statistically significant association between the levels of random and fasting blood sugar and the severity of diabetes.

HBA1C levels-

The mean HBA1C level for our study population was 9.49%. The normal upper limit is taken to be 6.3%. As the HBA1C level indicates the state of blood sugar control for a prior three months, this indicates a very poor control of sugars. This result only reinforces the conclusion in the previous section.

However, there was no significant relationship between HBA1C levels and the severity of DR. And no general trends were observed.

Conclusion

Serum VEGF levels were found to be significantly elevated in our study population when compared with the normal VEGF levels. This elevation of VEGF was observed in the entire diabetic population irrespective of whether they had retinopathy or not.

There was no significant relationship between the serum VEGF levels and the severity of DR.

Anemia was associated with severe grades of retinopathy.

There was an elevation of urea and creatinine levels in the study population; this elevation was higher in the more severe grades of DR.

Random blood sugar and fasting blood sugar levels were high in the study population indicating a state of chronic hyperglycemia and also suboptimal control of sugars.

The trend in HBA1C levels mirrored that of random and fasting blood sugars again highlighting a poor control of sugars.

In conclusion, an elevated serum VEGF level is significantly associated with diabetes mellitus and is linked to many of its complications. Though, there is much we are yet to discover about VEGF and its exact role in the pathogenesis of diabetic retinopathy one thing is certain, in VEGF lies a potential treatment and preventive strategy of not only diabetic retinopathy but also of many of the complications of diabetes.

Limitations

Many of the parameters studied showed wide standard deviations. This could be because of a small sample size. Possibly due to this same reason many of the outcome measures did not show a statistically significant relationship with the severity of DR. Therefore, by increasing the sample size it is possible we could have obtained more significant results.

We did not have a population of healthy controls. Hence, we could only compare our study population with known normal ranges. Having a group of age and sex matched healthy control could have eliminated any confounders which might have arisen.

We did not study other co-morbid conditions such as blood pressure, obesity, hypercholesterolemia. All these factors have been proven to affect the complications of diabetes and they would have made for useful study.

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ANNEXURE

Annexure 1.

STUDY PROFORMA

1) AGE :

2) SEX :

3) DURATION OF DIABETES :

4) COMORBID CONDITIONS :

5) LOCAL EXAMINATION OF EYE :

FINDINGS	RIGHT EYE	LEFT EYE
LIDS		
CONJUNCTIVA		
CORNEA		
ANTERIOR CHAMBER		
IRIS		
PUPIL		
LENS		
VISION		
TENSION		
OCULAR MOVEMENTS		
FUNDUS		

6) RESULT OF VEGF LEVEL IN BLOOD (ELISA METHOD):

Annexure 2.

CONSENT FORM

I have been explained, in a language that I can understand, about the study being conducted. I understand that this study is being conducted purely for research purposes. All that is required is 5 ml of blood which will be drawn only once. I am aware that I have the freedom to choose not to participate and my decision will have no bearing on my treatment.

All my reports and results will be maintained with strict confidentiality.

Patient signature

Place

Date

Annexure 3. RAW DATA

NO DIABETIC RETINOPATHY

S.NO	NAME	AGE	SEX	OP NO.	IP NO.	VEGF LEVEL	DURATION (YEARS)	HB %	UREA (mg/dl)	CREATININE (mg/dl)	HBAIC%	RBS (mg/dl)	FBS (mg/dl)
1	DHANDAPANI . S	55	M	O09004308	I14032002	702.175	6	14.4	22	0.87	11.1	297	161
2	THULSIAMMAL.P	72	F	O13035389	I14032947	853.228	7	11.1	16	0.49	7.52	151	175
3	ABDUL RAHIM.H	82	M	O14080159	I14033601	466.2252	1	14.5	13.55	0.6	7.9	182	165
4	CHELLAMMAL.N	55	F	O14081811	I14034441	633.9442	8	11.4	16	0.3	5.93	157	93
5	PALANISAMY.R	56	M	O15001404		705.0335	2	11.6	19	0.57	9.9	291	207
6	NARAYANASAMY	68	M	O10047831	I15001859	341.1629	2	13.3	18.22	0.85	8.24	205	158
7	ARUNMUGAM.S.L	65	M	O150010792	I15004869	1100.048	1	12	17.29	1.04	6.34	230	133
8	ARUCHAMY	50	M	O15025319	I15011508	31.44165	3	11.9	26	0.61	7.8	217	119
9	NAGARATHINAM	61	F	O98032390	I15012557	812.982	10	11.36	17	0.59	11.3	347	126
10	LAKSHMI.M	45	F	O15027025	I15012403	1034.881	1	11.4	31	1.38	9.34	208	248
11	SAKTHIVEL.R	59	M	O14045618	I15012825	793.6378	25	13	29	1.01	9.3	333	277
12	SHANTHA.P	48	F	O14089196		505.3543	5	11.7	16	0.4	9.3	204	215
13	MYILATHAL	45	F	O15029446		580.1847	1	11.2	24	0.57	7.8	64	72
14	PALANISAMY.V	53	M	O15006732	I15012112	581.9402	10	7.5	92	3.5	8.01	222	159
15	JAYAPRAGASAM.S	66	M	O15029035	I15013205	168.8314	16	15.3	14	0.88	6.98	223	135

MILD NPDR

S.NO	NAME	AGE	SEX	OP NO.	IP NO.	VEGF LEVEL	DURATION (YEARS)	HB %	UREA (mg/dl)	CREATININE (mg/dl)	HBAIC%	RBS (mg/dl)	FBS (mg/dl)
16	SUBBULU.R	72	M	O14068986	I14031004	403.4314	10	8.9	27.57	4.24	5.7	163	132
17	MANI.C	50	M	O15001719	I15001508	453.3152	20	9.3	12.02	0.95	7.71	362	151
18	PALANISAMY.S	65	M	O15010964	I15004873	1000.285	10	11.6	61	1.9	9.9	407	248
19	PADMAVATHY.S	55	F	O15012120	I15005403	196.2381	6	12.7	23	0.55	11.78	361	265
20	KRISHNAVENI	48	F	O15009271	I15004281	956.1171	15	10.8	42	0.55	11.38	274	139
21	BANNARI.S	45	M	O15012531	I15005657	485.0806	4	14.4	15	0.76	13.9	181	159
22	GOVINDASAMY.K	69	M	O15016138	I15007383	444.5504	12	9	31	0.57	8.3	123	86
23	JAMES	58	M	O140011081	I14004500	509.3276	20	12.9	32	0.65	6.1	142	124
24	CHANDRASEKAR	58	M	O12000557	I15012650	674.3131	25	11.7	31	1.07	10.37	253	128
25	GOKILAMANI.P	60	F	O15020910	I15009536	686.2036	1	9.8	58	1.87	10.06	296	290
26	THIRUMOORTHI	65	M	O13009631	I15013554	1296.156	36	8.9	69	2.91	6.35	229	96
27	MUTHUSAMY	64	M	O11076842	I15014123	738.3067	3	14.6	48	1.12	12.42	251	213
28	VEERAI.V	67	M	O15019783	I15014475	451.136	8	12.6	16	0.94	12.5	261	386
29	MANIMEGALAI	55	F	O15037100	I15016935	1190.554	15	7.7	27	1.11	11.74	287	110
30	GOVINDARAJ	47	M	O15031530	I15014258	507.3442	7	14.8	16	0.72	7.63	146	148

MODERATE NPDR

S.NO	NAME	AGE	SEX	OP NO.	IP NO.	VEGF LEVEL	DURATION (YEARS)	HB %	UREA (mg/dl)	CREATININE (mg/dl)	HBAIC%	RBS (mg/dl)	FBS (mg/dl)
31	MARATHAL.M	65	F	O14081044	I14034062	505.3543	4	11.4	11.21	0.47	11.76	303	167
32	RAMASWAMY	50	M	O14082745	I14034990	693.5164	8	10.5	37	1.25	10.74	220	196
33	THANALAKSHMI.G	64	F	O13071937	I14035057	519.1464	10	12.2	7	0.4	11.42	210	186
34	SOWKIT ALI	55	M	O15010400	I15004689	461.9528	2	6.9	49.59	1.14	5.9	193	121
35	DURAISAMY.P.S	49	M	O15005411	I15005133	612.6858	1	15.8	22	0.76	9.29	176	191
36	PALINISAMY.R	58	M	O15000162		374.3529	10	9.9	128	4.77	7.19	176	191
37	RAMASWAMY.C	70	M	O15011471	I15005131	848.7637	15	11.4	28	0.8	8.5	263	207
38	GOVINARAJU	50	M	O14043599	I15005529	294.7695	3	15.1	18	0.74	10.23	119	126
39	ABUL KATHAR.K.S	70	M	O15009721	I15006526	671.3031	7	14	23	0.5	9.8	255	165
40	SARASWATHY	56	F	O10092419		286.194	20	10.2	39	1.24	9.1	102	81
41	SELLAMMAL	65	F	O15009725	I15006832	1119.566	10	7	52	1.04	11.2	189	158
42	RAJENDRAN	52	M	O15018124	I15008335	743.676	10	12	47	2.02	10.32	296	217
43	VELUSAMY.P	55	M	O0019018	I15008677	61.5702	19	14.2	25	0.66	10.04	287	137
44	MANOHARAN		M	O06065460	I06031533	677.308	12	10.5	28	1.2	11.02	202	140
45	GOVINDAN.K	67	M	O15015004	I15006909	314.312	10	12.8	26	0.88	7.56	120	134

SEVERE NPDR

S.NO	NAME	AGE	SEX	OP NO.	IP NO.	VEGF LEVEL	DURATION (YEARS)	HB %	UREA (mg/dl)	CREATININE (mg/dl)	HBAIC%	RBS (mg/dl)	FBS (mg/dl)
46	MUTHUKUMARASAMY	42	M	O12053890	I14033043	665.2369	12	12	14.02	1.21	11.84	282	173
47	PADMANABHAN.A	60	M	O14078228		428.895	5	10	26.17	2.93	6.5	196	125
48	MANI.R	47	M	O14080175	I14033629	751.6404	10	9.8	35.98	0.98	15.05	228	196
49	GANAPATHI.P	54	M	O14084535	I14035773	856.5545	8	12.3	28.79	0.81	12.01	388	95
50	RAMACHANDRAN	61	M	O14085841	I14036283	8.948788	5	15.1	14.99	0.89	9.85	215	225
51	SUBRAMANIAN	60	M	O08079840	I15004440	113.7779	20	11.4	134	3.64	12.2	263	120
52	PADMANABHAN.P	60	M	O15023416	I15010628	401.0616	10	10.9	52	1.07	15.8	361	244
53	SHANMUGAM.K	70	M	O15026484	I15012142	904.3442	15	9.1	19	0.93	7.6	130	162
54	ANAND.S	54	M	O15027347	I1501209	602.6125	8	12.1	25	0.7	8.86	239	167
55	GOPAL.G	65	M	O15026970	I15012370	831.718	15	11.8	31	0.84	5.39	148	133
56	MANIVENTHAN.S	47	M	O14085698	I14036238	241.3616	4	9.8	33.64	3.7	5.77	178	127
57	RAJAMANICKAM.A	58	M	O15002053		244.4577	12	11.2	38	0.57	9.2	321	294
58	ROCHAYAN	52	M	O15016252	I15007458	702.175	9	8.2	17	0.6	15	190	202
59	KALAMANI	64	F	O10035485	I15015630	779.9649	30	9.4	21	0.79	11	125	122
60	VIJAYAN	45	M	O08017551	I15018263	428.695	16	11	17	1.1	9.1	180	110

PDR

S.NO	NAME	AGE	SEX	OP NO.	IP NO.	VEGF LEVEL	DURATION (YEARS)	HB %	UREA (mg/dl)	CREATININE (mg/dl)	HBAIC%	RBS (mg/dl)	FBS (mg/dl)
61	SHANMUGAM.V.M	61	M	O12093838	I14032919	117.611	11	11.4	42	1.26	11.14	155	133
62	GIRIJA	64	F	O14034590	I14035236	325.198	10	10.3	59	1.32	10.1	200	291
63	DHANUSKODI.F	41	M	O13022843	I15005699	693.516	9	12.8	31	0.61	11.6	312	110
64	KALIMUTHU.P	74	M	O15011620	I15005151	455.486	14	8.4	100	3.24	13.2	251	133
65	RENUKA DEVI	57	F	O11089002	I13029734	341.163	18	11.8	38	0.98	7.8	207	143
66	RAMASAMY.M.V	64	M	O14074976	I15006925	489.191	3	11.3	44	1.96	7.2	183	111
67	NAGALINGAM.K.S	52	M	O15020630	I15009400	262.704	12	10.4	43	2.23	7.7	194	142
68	MOHAN.S.N	55	M	O08061190	I15010893	1029.94	15	11.2	56	1.96	11.1	261	198
69	RAJAMANI.A	55	M	O150050322	I15011040	478.862	8	14.3	25	0.53	10.6	286	235
70	PANCHAKALYANI	65	F	O14084955	I14035872	779.965	1	8.9	112	2.99	8.49	224	117
71	GOVINDASAMY.M	70	M	O96000653	I10015283	464.093	20	12.4	19	0.81	7.5	186	118
72	MARIMUTHU.K	65	M	O12035212		396.294	20	11.4	31	1.7	7.8	145	109
73	MUTHUSAMY.M	57	M	O14040537		196.238	4	10.6	64	2.59	9.6	185	104
74	SUKUMAR	42	M	O13074323	I15018487	271.621	6	12.3	63.6	11.63	6.9	150	108
75	PAPPATHY	56	F	O14052771	I14021935	1145.32	3	11.9	10	1.6	9.2	190	133